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PHOTOMORPHOGENETIC EFFECTS OF REGIONS IN THE VISIBLE SPECTRUM ON CERTAIN PLANT SPECIES

by

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ABSTRACT

Photomorphogenetic effects of regions in the visible spectrum on certain plant species were studied. A spectrophotometric method for estimation of IAA was developed. A relationship was found between the IAA content in bean plants after 1 and 7 light cycles and the growth pattern after 7 cycles. Plants grown in the dark were the tallest and contained the most IAA. Of the light treated plants those exposed to blue were the tallest and had the highest IAA content. Red light treatment produced the shortest plants, with the least IAA. Application of IAA did not reverse completely the inhibitory effect due to red light. When labelled IAA was applied more was recovered from the blue than from the red treated plants. Red light reduced the length of the first internode in corn and increased the chlorophyll content in normal barley. In addition to reduction in endogenous IAA levels in beans, red light also reduced elongation of hypocotyl, epicotyl and petiole, dry weight of shoots and cotyledons, and total nitrogen in leaves. On the other hand, red light increased chlorophyll and carotene content, leaf size and dry weight, and root formation. These trends were somewhat reversed in treatments containing a higher proportion of far-red, which implicates the phytochrome system. However, many responses were similar in the blue and red-far-red indicating that phytochrome may also absorb in the blue or was not the only photoreceptor responsible for the photomorphogenetic effects.

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INTRODUCTION

All life on this planet is dependent either directly or indirectly upon light. In addition to supplying the energy for plant life light controls a number of morphogenetic mechanisms. These light controlled reactions require relatively small amounts of light energy to induce large effects. Since most of the photo-responses of plants are governed by the visible spectrum (380 - 760 mμ), it is important to consider the effects of different wavelengths within this range upon plant growth.

Over the past 150 years there have been numerous studies conducted on the effects of light quality on plant growth and development. These studies have produced many controversial results especially with regard to elongation (52). The validity of some of this early work has been questioned (73) due to the inability to control the experimental conditions precisely. Even with techniques which provide more precise control of intensity and spectral region, there still appears to be no agreement on some of the photomorphogenetic responses. It has been suggested that some discrepancy in results could be due to differences in experimental methods (52) or materials (72).

A preponderance of the experiments pertaining to IAA and light quality have involved the use of sections from different plant species under a variety of experimental conditions. Thus the role of IAA and other growth substances in the photomorphogenetic control of growth by light is in a state of confusion.

This work was therefore undertaken to investigate the relationships between the endogenous free IAA levels and elongation of plants exposed to different wavelengths, and to study the metabolic fate of exogenously supplied IAA. In addition, this study includes data obtained on a number of other photomorphogenetic responses of plants.

LITERATURE REVIEW

A great deal of work has been done on the effects of light quality on plant growth and development and there are excellent reviews on the subject (10, 55, 80). A complete review of the extensive literature is beyond the scope of this thesis. It will therefore deal only with those effects of light quality on plants which have been the subject of this study.

Germination

Work conducted on the relative effectiveness of various regions of the spectrum upon germination has been adequately reviewed (10, 15, 17, 68). Results obtained as early as 1883 by Cieslar and in 1907 by Kinzel have been cited by Crocker (15). Both these workers found that longer wavelengths stimulate and shorter wavelengths inhibit germination.

More accurate and extensive work conducted with Lactuca sativa was reported by Flint and McAlister (21) in 1935. Their results showed that wavelengths between 440 and 480 mμ were inhibitory and between 520 to 700 mμ were stimulatory to germination. They also observed that there was a sharp break in the response curve at 700 mμ, with wavelengths shorter than this being very promotive and those immediately longer being completely inhibitory to germination. They also found that there was good agreement between the action spectrum and absorption spectrum of the acetone extract of Lactuca seeds, which in turn is nearly identical with the absorption spectrum of chlorophyll. On this basis they concluded that chlorophyll was probably the pigment absorbing the light responsible for seed germination.

The observations of Flint and McAlister for wavelengths greater than 520 mμ were confirmed by the Beltsville group (12). In extending their experiments they observed the possible existence of a reversible reaction in germination control. An action spectrum for both promotion and inhibition of germination of Lepidium virginicum (67) and Lactuca sativa (12) was determined. There was an occurrence of maxima for promotion near 660 mμ and for inhibition near 740 mμ. This red-far-red response was also present in the case of Henbit seeds, Lamium amplexicaule (40). The work of Wareing (77) lends further support to the red/far-red response of seeds to germination. He found with Nemophila seed that germination is promoted by red and is inhibited not only by a far-red region but also by blue light.

In an attempt to locate the photoreceptor, Ikuma and Thimann (38) found that red light promoted germination of lettuce seed (Grand Rapids) and far-red is inhibitory only when the hypocotyl half of the seed is exposed to light. They concluded that the photoreceptor is probably located in the tip of the hypocotyl.

It has been reported that germination can be promoted by treatment with gibberellin (41) or kinetin (53) and this effect is apparently not reversed by far-red light treatment. Likewise, Haber and Tolbert (28) have demonstrated that gibberellin and kinetin have distinct effects on the germination of Grand Rapids lettuce seed, and that the treatment with either of these chemicals cannot substitute for red light treatment under selected conditions.

Evenari (17) has pointed out that there are similarities in the action spectrum for photoblastism (the influence of the presence or absence of light on the germination of some seeds) and the action spectra of other light conditioned processes.

Elongation

Reports in older literature which include those of Popp (60) and Shirley (65) concluded that the blue-violet end of the spectrum was necessary for normal vigorous growth of plants and that the red end of the spectrum promoted stem elongation while the blue-violet end checked it. The results of these earlier workers have been criticized by Vince et al. (73) on the basis that the cut-off of the filters used, particularly at the blue end of the spectrum, was not sharp so that wave band limits were ill defined and because of differences in percentage transmission of the filters, energy levels in the different wavelength regions were unequal.

In recent years a number of studies have been conducted using improved equipment. In spite of this a great deal of confusion still exists on the subject of light quality and its effect upon elongation (52). Some European workers have suggested that the relative effectiveness of blue and red radiation in determining final internode lengths varies according to the experimental technique. In their opinion intensity is a very important determining factor. Fortanier (22) concluded from his studies that low intensities of blue and infra-red radiation have an elongating effect, contrary to the effect of high light intensities. These findings have been confirmed by Meijer (52)

who found with all the species he studied that red light was more effective in inhibiting internode elongation at low energy levels but blue light was more effective than red at high energy levels. According to Wassink and Stolwijk (79) plants growing exclusively in monochromatic light respond to wavelength in a way markedly different from that shown by plants receiving supplementary light at the same intensity after a daily period in white light.

It seems likely that many of the differences observed may be due to variation in the response of the species grown rather than to any other factor (72). In experiments with Pisum and Lycopersicon, Vince and Stoughton found that the response was different under identical conditions. They also found that two varieties of Pisum sativum were different in their response. From their results they concluded that different species and even varieties of the same species do not respond in the same way when grown under similar conditions in light of different wavelengths.

There is evidence that the response of various organs to light depends on the stage of development of those plants at the time the light is given. Thompson and Miller (66) in their work with peas report that the morphogenetic effect of light is limited to the later stages of organ growth.

A number of workers (4, 26, 39) found that red light was more effective than other light in inhibiting the growth in length of the first internodes in Avena. Action spectra for inhibition of the first internode of Avena (81) and for Hordeum (11) have been worked out.

The significant features of these action spectra were maximum effectiveness of light in the region of 600 to 650 mμ and an abrupt decrease at both longer and shorter wavelengths. Weintraub and Price (81) working with 12 species of monocotyledons other than Avena and Hordeum and representing eight different tribes, found that a given inhibition required the order of 1,000 times the energy when violet (435 mμ) was used instead of red (623 mμ). An action spectrum on the effect of light quality on a dicotyledon (Red kidney bean) has been worked out by Downs (16). He found that the maximum effectiveness for inhibition of hypocotyl length was 640 mμ and 730 mμ for the opposing far-red action.

Root Formation

Relatively few studies have been conducted on the effect of light quality on roots. Went and Thimann (82) reported that illumination at any wavelength decreased lateral root initiation in etiolated pea stem cuttings. They thought that this was brought about by decreasing the effectiveness of the applied IAA on the root initiation process. From a study with peas Torrey (69) observed that maximum root elongation and lateral root formation occurred when the roots were maintained in the dark. His conclusions were that red light inhibits lateral root formation much more effectively than blue or green light. Humphries (36) observed that a 16 hour photoperiod of incandescent light given before or after excision of the bean hypocotyl decreased root number and total root fresh weight of low-boron plants. Since this was not the case in plants irradiated with fluorescent light, which is lower in emission of far-red, he hypothesized a connection between radiation in the red or far-red part of the spectrum and inhibition of root growth when boron is a limiting factor.

A more detailed study using excised wheat roots was done by Bjorn et al. (8). Their results do not agree with those of Torrey (69) who found that red light inhibits branching in pea roots. They worked out an action spectrum for both cell multiplication and cell elongation in excised wheat roots. They found that inhibition of multiplication had a peak around 400 mμ whereas red light had little effect. The action spectrum for the inhibition of cell elongation had two peaks of approximately the same height near 430 mμ and 650 mμ.

Leaf Expansion

In a recent review on the physiology of leaf growth Humphries and Wheeler (37) dealt with some of the aspects of light quality and leaf expansion. Light is necessary to promote leaf expansion and Parker and Went (59) in their studies with Alaska pea, found that maximum expansion occurred in red light. Downs (16) conducted similar experiments with red kidney bean and showed that the expansion of intact leaves that occurred in red light could be reversed by far-red light. Using leaf discs of the dwarf stringless greenpod bean, Liverman et al. (48), showed that the red-far-red system operated in a similar fashion and reported that the light requirement for expansion of leaf discs and for the intact leaves were identical. Liverman (47) also points out that the action spectrum for leaf growth is almost precisely that found for lettuce seed germination.

Klein et al. (44) found that the wet weight of bean leaves increased at a rate which was approximately proportional to the logarithm of the incident radiance and that the 630-700 mμ red band was much more effective than the 710-725 mμ far-red band.

Klein and Wansor (43) studied the effects of visible and ultraviolet radiant energy on the expansion of bean leaf disks. They found that red and blue were equally effective in inducing expansion and this effect was negated by far-red. They also found an interaction between red and blue. The order blue-red was more effective than either red + blue or red followed by blue. Ultraviolet radiation was also effective in inducing leaf disk expansion. Red, blue and ultraviolet irradiations all induced disk enlargement by cell multiplication.

Chlorophyll Production

Appleman (2) in a study of barley seedlings found that chlorophyll accumulates at approximately the same rate in red and blue grown plants during the first two days. After that there is no further accumulation in the plants grown in red light, however, the plants grown in blue light continue to accumulate chlorophyll until the fourth day when a maximum about 33% higher than that in red light is reached.

It has been reported by Withrow et al. (85) that chlorophyll formation requires only a low energy light reaction. Pretreatment of leaves with red light followed by a dark period of 5 to 15 hours resulted in the elimination of the latent period in subsequent chlorophyll formation, with some indication that the red induction might be reversed by an exposure to far-red. Virgin (74) showed that red light pretreatment is much more effective than blue light. In a more recent study Virgin (75) worked out the action spectrum for the elimination of the lag phase in chlorophyll synthesis in wheat leaves. The spectrum showed a maximum at 660 mμ with minor shoulders

at around 540 mμ, 600 mμ and possibly at 700 mμ. That the red-far-red response was operative in chlorophyll synthesis is evident from the work of Price and Klein (61). In their studies the induced stimulatory effect of a red pretreatment was nullified by a subsequent exposure to far-red administered either immediately after the red induction exposure or even after the interposition of several hours of darkness. These findings are in general agreement with those of Mitrakos (54) who found that the red effect on chlorophyll synthesis could be lowered with subsequent far-red exposure by approximately 30%. He concluded that the last given light quality determines the quantity of chlorophyll formed.

Photosynthesis

In 1937 Hoover (35) measured an action spectrum for the rate of photosynthesis. He obtained a two peaked curve for relative photosynthesis in wheat. His measurements indicated that blue and red light were more efficient than light in the yellow green region. When the curve was replotted to indicate relative quantum efficiency, Burns (14) found that blue light was even slightly more effective than red. Relative efficiency in the green (550 mμ) was about 50% of that in the blue violet around 450 mμ. Monochromatic light curves for the rate of photosynthesis against intensity have been determined for Sinapis alba by Gabrielsen (23). At low intensities the rate is maximal in red and falls to a minimum in blue and as the intensity is raised the same saturation yield is approached at all wavelengths.

There are a number of reports (71, 78) which indicate that the relative dry weight gain is greater in the red than in the blue.

On the contrary Moshkov (56) reports reduced dry weight of plants grown in red light as compared with those grown in equienergetic blue or green light. But Wassink and Stolwijk (80) are of the opinion that this is almost certainly due to a large amount of far-red radiation contaminating the red light.

Interesting work has been done on the effects of light quality on the products of photosynthesis. This work has been done mainly by Russian investigators and their findings have been cited by Tregunna et al. (70). It has been reported that leaf disks of corn, sunflower and bean leaves have added more dry matter during photosynthesis in red light than in blue light. The percentage of proteins in the dry weight added under illumination with blue was higher than that with red. In tobacco leaves, red light was reported to increase incorporation of total $C^{14}O_2$ into the organic acid fraction, while blue increased radioactivity in the amino acids. Chromatographic separation of the amino acids indicated that the increase was due to alanine and aspartic acid. While the blue-violet end favoured protein synthesis, the longer wavelengths favoured the synthesis of carbohydrates. However, Tregunna et al. (70) found no effect of wavelength on the distribution of absorbed CO_2 between the ethanol-soluble and insoluble fractions. There was no evidence of red light stimulating the synthesis of sugars. Red light when compared to white increased incorporation of CO_2 into glycine but had no effect on serine. Blue brought about a considerable decrease in glycine and some decrease in serine. From these studies it is evident, however, that the distribution of carbon among the products of photosynthesis is affected by the quality of light.

Indoleacetic Acid

Studies on the relationships between light and indoleacetic acid in plants have produced confusing results. In general green plants require light for auxin formation and light also brings about auxin destruction, more strikingly in etiolated plant material than in green plants (Leopold, 45).

Van Overbeek (58) in 1936 found that exposure of etiolated Avena seedlings to 3 hours of yellow-red light (no wavelength shorter than 570 mμ) reduced the diffusible auxin content of the coleoptile tips to about 65 per cent of that of the dark controls. More recent work by Blaauw-Jansen (9) and Briggs (13) has shown that red light lowers the endogenous level of IAA in plants. On the other hand, Galston and Baker (24) reported that IAA can be photoxidized by blue light in the presence of riboflavin. Hillman and Galston (34) have also reported that the activity of IAA oxidase was inductively inhibited by red light. The inhibition was reversible by near infrared radiation. Meijer's (52) studies indicated, however, that blue and far-red light increased auxin content. In his studies with gherkin seedlings he found that application of synthetic auxins produced elongation in plants inhibited by red light. This effect was reversed by the application of anti-auxins. From these findings he suggested that IAA is involved in the effect of light on plant elongation. From a study conducted with pea internode sections, Hillman (33) concluded that radiations act primarily on a component of endogenous growth which is distinct from IAA or gibberellic acid induced growth. By using similar plant material Bertsch (7) found that both IAA and gibberellic acid reduced or eliminated photoinhibition caused by either red or blue light.

In view of what appears as conflicting reports and insufficient evidence, Heath and Vince (30) in a recent review, were tempted to conclude that IAA is not directly related to the inhibition of growth by red light.

In a recent review Galston and Hillman (25) draw attention to the fact that the products of IAA photodegradation are not completely known. In the riboflavin sensitized system the appearance of indole-aldehyde has been demonstrated along with other unidentified products. The reaction is as follows:

$$\text{IAA} + O_2 \longrightarrow \text{indolealdehyde} + CO_2 + H_2O$$

The degradation is not confined to the side chain but eventually gives rise to unknown products by opening the ring structure itself. Galston suggests that from the physiological point of view, the initial decarboxylation appears to be the most significant.

Phytochrome

It is evident from this review that a number of photomorphogenic responses are controlled by the red-far-red effect of light. Therefore, a review on the effects of light quality on plant growth and development would not be complete without the mention of phytochrome. A recent paper (32) of the Beltsville group points out that the reversible reaction of phytochrome which is as follows

$$P_{660} \underset{\text{730 mμ or darkness}}{\overset{\text{660 mμ}}{\rightleftharpoons}} P_{730}$$

controls many aspects of growth and development in higher plants. Among the factors controlled by phytochrome are: flowering, stem elongation

(etiolation), leaf movement and expansion, plastid formation, seed germination, anthocyanin production and bud dormancies.

However, Mohr (55) thinks that phytochrome cannot be the only photoreactive system in photomorphogenesis. In his studies he observed that certain reactions were achieved by irradiating with high energy for a relatively long period of time. This system is called the "high energy reaction". The action spectra of the high energy reaction show peaks in the blue and in the far-red range of the visible spectrum.

In spite of the numerous studies that have been conducted on photomorphogenic responses and the knowledge being accumulated on the phytochrome system, Liverman (46) suggests that it would be difficult to explain all these different responses on the basis of a series of identical reactions. The task of explaining for the various photo-responses the cause-effect relationship between the primary photo-reaction and the final response still remains (Mohr, 102).

MATERIALS AND METHODS

Description of Growth Cabinets

The main features of the light-tight growth cabinets, which were of plywood construction, are shown in figure 1. The all-plastic filters were fitted onto the top of the cabinets. Each cabinet was equipped with two air ducts, and the inlet at the base had an adjustable shutter to control air circulation. To maintain a uniform temperature of 20 $\pm$ 1 C, warm air was forced over a refrigeration unit through the ducts. The cabinets were painted glossy white inside and had an adjustable shelf.

The light assembly (fig 2) was placed over the filters and had adjustable lamp racks. To cool the lamps air was taken in at the base of the lamp housing and drawn out at the top.

The characteristics of the light filters used have been reported earlier by Zalik and Miller (86). In this study the range of wavelengths obtained in the growth cabinets by these filters will be referred to by color as shown in table I.

The incident light intensities were adjusted to provide uniform energy in each cabinet. Measurements were made by means of an Eppley pyrheliometer of the 180° Weather Bureau type used in conjunction with a Rubicon type B potentiometer having a sensitivity of 1 microvolt.

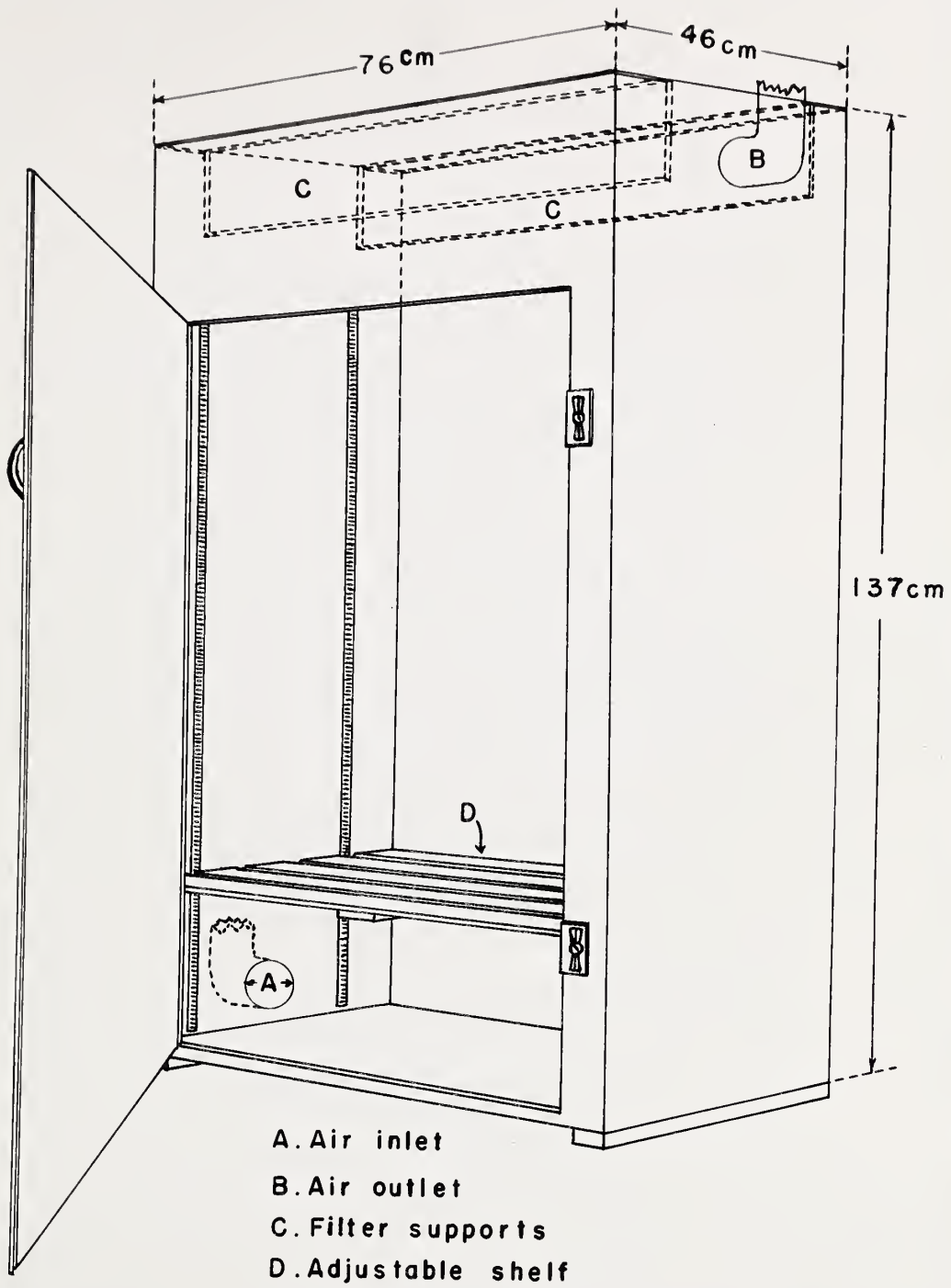


FIG. 1. Growth cabinet.

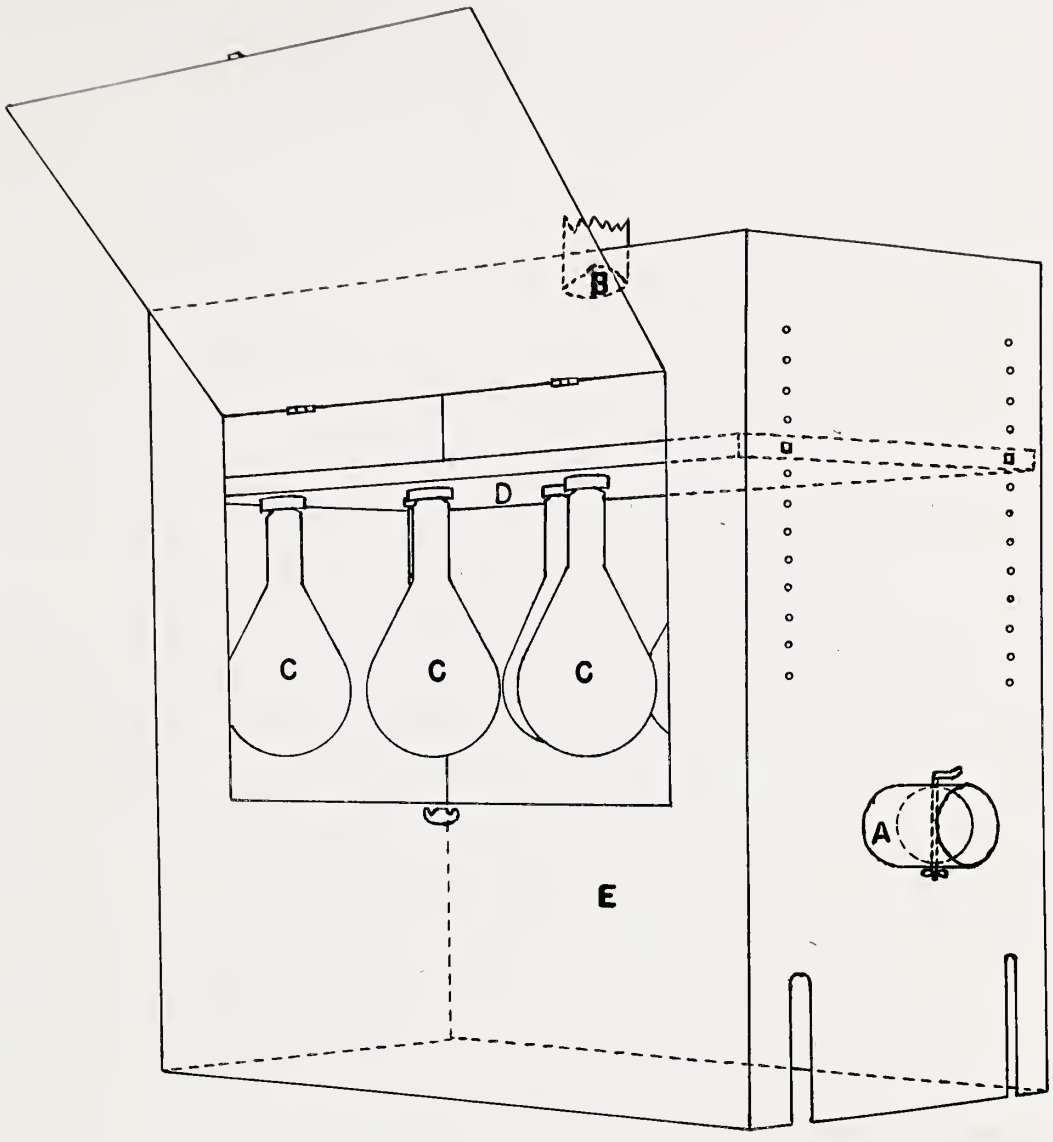


FIG. 2. Light assembly.

Table I

Transmittance Data and Color Designation for the Growth Cabinets

Wavelength mμ at		Color
Peak	50% transmission	
-	Control	Dark
426	393 - 463	Blue
493	460 - 532	Green
532	500 - 577	Yellow
560	540 - 605	Orange
600	580 - 655	Red
636	610 - 705	Deep-red
680	646 - 738	Red-far-red
-	346 - 890	White

Since the blue cabinet was the one of limiting light intensity, the pyrheliometer was used initially in this cabinet and a reading of 10.4 μv was obtained. This value corresponds to 2900 ergs/cm^2/sec as derived from the following equation using 2.5 millivolts/cal cm^{-2} min^{-1} as the calibrated value of the pyrheliometer.

$$\frac{\text{ergs}}{\text{cm}^2\ \text{sec}} = \frac{4.185 \times 10^7\ \text{ergs/cal} \times 10^{-3}\ \mu\text{v/millivolt}}{\frac{2.5\ \text{millivolts}}{\text{cal cm}^{-2}\ \text{min}^{-1}} \times 60\ \frac{\text{sec}}{\text{min}}}$$

$$= \frac{4.185 \times 10^7 \times 10^{-3} \times 10.4}{2.5 \times 60}$$

$$= 2900\ \text{ergs/cm}^2\text{/sec}$$

The light intensities in the other cabinets were adjusted to this value by adjusting

(1) the height of the shelves in the cabinets

(2) the height of the lamp racks

(3) and the wattage of the lamps.

Germination Studies

Paper towels were cut into circles of 9 cm diameter with a 2 cm flap at one end. Two of these circular papers were moistened and placed into 9 cm petri dishes. These dishes were placed into the covers which contained distilled water. The flap served as a wick and kept the paper constantly moist. Fifty seeds of freshly collected stinkweed (Thlaspi arvense L.), red-root pigweed (Amaranthus retroflexus) and one month old buckwheat (Fagopyrum tataricum) were placed on the moist paper and the dishes were covered. Duplicate dishes were placed in each of the cabinets kept at a photoperiod of 12 hours light and 12 hours darkness. Germination counts were made after 8 days. Seeds whose radicle had emerged more than 1 mm were considered germinated.

Growth Measurements

Seeds of a number of species were germinated to select species which had uniform germination and could grow well under relatively low light intensity. Of the two species found to be suitable one was a dicotyledon, Phaseolus vulgaris L. (var. Dutch brown beans) which was obtained from Holland through the Peter Groot Seed Co., Edmonton, Alberta. The other was a monocotyledon, Zea mays (var. Altagold corn) obtained locally.

Ten seeds each of both beans and corn were planted in 6 inch plastic pots containing California mixture (5). To each pot 180 ml of water was added.

The pots containing bean seeds were placed in the dark for five days and the temperature maintained at 25 ± 1 C. After 5 days in the dark, the seedlings were 4 to 5 cm tall. The seedlings were thinned to 4 or 5 uniform plants per pot and were then moved to the growth cabinets and exposed to different wavelengths of light with a photoperiod of 8 hours light and 16 hours darkness. This photoperiod was used in all studies involving beans. After 7 days the length of the hypocotyls, epicotyls and petioles of 13 plants from each treatment were measured. Leaf area was measured in sq cm by tracing the outline of the leaf on squared paper. The dry weight of the plant parts were obtained by drying them to constant weight in an oven at 100 C.

In order to determine the region of elongation and observe whether there were any relative differences in the various wavelengths, the bean seeds were allowed to germinate in the dark for 7 days. These dark-grown seedlings which were 10 to 11 cm tall were marked with India ink, into ten 1-cm segments. They were then moved into the growth cabinets and after radiation for 3 days the elongation occurring in each segment was measured.

In the study with corn the pots were placed directly in different growth cabinets. A photoperiod of 12 hours light and 12 hours darkness was employed in this study. After 12 days of irradiation, the length of the internodes, coleoptiles, leaves and the dry weight of shoots and roots were determined.

Rooting Studies

Bean seeds were germinated according to the method described for elongation studies. The seedlings were excised 4 cm below the cotyledons and were immersed in brown bottles which were covered with aluminum foil and contained nutrient solution. The nutrient solution containing 10^{-3} g/litre boric acid as described by Hemberg (31), was used. The bottles were transferred to the growth cabinets. After 5 cycles the number of adventitious roots was counted.

In another experiment 5-day old dark grown bean seedlings were exposed to 1 cycle of light. They were excised and immersed in nutrient solution as before. One set was transferred to the white cabinet and the other set to the dark cabinet. In the set which was transferred to the dark the nutrient solution also contained 2% sucrose. After 5 days the number of lateral roots were counted.

Pigment Determinations

The chlorophyll and carotene content in leaves were determined for the following.

(a) Leaves of Phaseolus vulgaris.

(b) Leaves of normal and mutant barley (var. Montcalm).

The mutant was a simple recessive yellow viable (yv) as described by Walker et al. (76). The barley seedlings were grown for 23 days in a photoperiod of 12 hours light and 12 hours darkness.

Duplicate samples of four leaves were selected at random from each treatment. They were harvested, weighed and cut finely

into glass mortars. The pigments were extracted from the leaves with a total of 35 ml of pure acetone by grinding them four times in a mortar with washed sand and $CaCO_3$. The extraction was done in the darkroom equipped with a green safelight having a Farrand interference filter #110323 (transmit. 498 mμ, half band width 12 mμ). The extracts were centrifuged at 10,000 g for ten minutes. The pellet was discarded and the volume of the clear extract measured. The absorption between 400 - 700 mμ of the clear extract in a 1 cm cell was measured using a Beckman DK1 spectrophotometer. The chlorophyll a and b content was estimated from the equation given by Maclachlan and Zalik (51) and the carotene content was estimated using the equation of von Wettstein (83).

Nitrogen and Amino Acid Determination

Five day old dark grown bean seedlings which were exposed for 5 days (8 hr light and 16 hr darkness) to the blue, green, deep-red, red-far-red, white light treatments and the dark control were used for this study.

Four leaves without petioles were harvested in duplicate from each treatment. They were homogenized with a Servall omnimixer for 5 minutes in 10 ml of distilled water at 45% line voltage. The homogenate was transferred into stainless steel centrifuge tubes and centrifuged for 15 minutes at 22,000 g in order to separate the particulate fraction. The supernatant was transferred to test tubes, and 5 ml of 5% trichloroacetic acid was added, and the reaction was allowed to proceed for 24 hours. In order to precipitate the acid-insoluble fraction the solution was centrifuged at 22,000 g for 20 minutes. The supernatant (acid soluble fraction) was poured into test

tubes and 1 ml of 5% trichloroacetic acid was added to check for completeness of protein precipitation. The above fractionation procedure was conducted in the cold room (4 C).

Nitrogen determinations were made on the three (particulate, acid-insoluble, acid-soluble) fractions by a microkjeldahl method (3).

An aliquot of the acid-soluble fraction was evaporated to dryness in a flash evaporator at 40 to 50 C, taken up in distilled water and evaporated again to dryness. The residue was dissolved in 5 ml of distilled water and the pH was adjusted to 2.2 with HCl. Another 5 ml of 0.2N Na-citrate buffer (pH 2.2) was added to make the final volume to 10 ml. An aliquot of this was used for amino acid analysis which was done by means of a Beckman/Spinco Model 120 Amino Acid Analyzer.

Measurement of Endogenous Indoleacetic Acid (IAA)

Bean seedlings were germinated in the dark as described for elongation studies. In order to minimize the number of estimations required and to reduce the overlapping of wavelengths, only blue, yellow, red, red-far-red and dark treatments were selected for IAA studies. Endogenous levels of IAA in the 5 day old dark grown seedlings were measured before and after 1 and 7 light cycles of 8 hours light and 16 hours darkness.

The method used for isolation and measurement of IAA has been reported earlier by Fletcher and Zalik (19, 20) and will be described in detail here.

Thirteen plants from each treatment were cut so as to remove 3 cm of the apical region (including cotyledons). These apical segments were immediately immersed in 25 ml of cold absolute methanol. They were then ground at 3 C in a Servall Omnimixer for 2 minutes at 45% line voltage, and allowed to stand at this temperature for 4 hours. The extract was then filtered and an aliquot of the filtrate representing 3 plants (approximately 2 g) was evaporated to dryness under vacuum at 40 C. The contents of the flask were taken up in 0.5 ml methanol and applied as a band on a 7.5 x 46 cm strip of Whatman No. 1 paper. Descending chromatography was used and the chromatogram developed for 16 hours, with isopropanol, ammonia and water (8:1:1). Two solvent systems were tried, one using 28% and the other 7% ammonia. After air drying the chromatogram, a 6 mm strip was sprayed with p-dimethylaminocinnamaldehyde (DMCA) to locate the IAA (29). The corresponding unsprayed portion of the chromatogram was eluted with 3.5 ml of methanol and the spectrum between 230 and 300 mμ measured by means of a Beckman DK1 spectrophotometer. Levels of IAA were determined from the absorption peak at 280 mμ using a standard curve (19). The identity of the endogenous IAA was confirmed by the Salkowski and Ehrlich reagents and by comparison with the R_fs and ultraviolet spectrum of synthetic IAA.

Levels of IAA were measured also by the first internode test developed by Nitsch and Nitsch (57). For the first internode test the IAA was eluted from the chromatogram with water in place of methanol. The hulless variety Vicar oats was used in the test, and the concentrations of IAA were determined from a standard curve for this variety.

Colorimetric Estimation of Synthetic IAA

An aqueous IAA solution was made up to contain 10.33 mg/l. Twenty ml of this solution was poured in 9 cm petri dishes. The apical 3 cm of 3 plants of 5 day old dark grown bean seedlings were immersed into one set of petri dishes containing IAA solution. Another set of dishes contained only IAA solution and served as control. Both sets of dishes were transferred into the various growth cabinets. After 8 hours radiation the IAA concentration in each treatment was estimated by the method of Gordon and Weber (27).

Metabolic Studies with IAA

Bean seedlings were germinated in the dark as described. Ten µl of aqueous IAA solution containing 100 µg IAA were applied to each plant by injecting 5 µl with a syringe into each cotyledon. The injected plants were exposed to 1 cycle of light. The plants were then harvested and the IAA extracted and measured by the spectrophotometric method described earlier.

The radioactive IAA labelled with C^{14} in the 2 position of the side chain was obtained from the California Corporation for Biochemical Research. An application of 0.2 µc per plant of radioactive IAA (specific activity 13.3 mc/mmole) was administered as above.

After 1 cycle some of the treated plants were pressed between blotting paper and dried in an oven at 40 C for 24 hours. Radioautograms were made with Ansco X-ray film. Film was exposed to the plants for 7 days and processed with Kodak X-ray developer and fixer.

Three intact plants were taken from each of the treatments and the IAA was extracted to yield three fractions. Fraction I, designated free IAA, was obtained by methanol extraction as already described except that homogenization was carried out at 60% line voltage for 3 minutes. The residue after filtration was further extracted with methanol overnight in a Soxhlet. This yielded fraction II, assumed to contain bound IAA. The resulting residue was hydrolyzed with 0.5N HCl for 3 hours to yield a filtrate, fraction III, which would contain more strongly bound IAA (6). The volumes of the three fractions were reduced to 3 ml in a flash evaporator at 40 C. A 0.1 ml aliquot from each fraction was plated on aluminum planchets and the radioactivity determined in a gas flow counter equipped with a micromil window and an automatic sample changer. The efficiency of the counter was 48% for samples having an activity of 5000 cpm.

Aliquots of 0.2 ml of each fraction were chromatogrammed. The chromatograms were scanned with a 4π chromatogram scanner. A permanent record was obtained by preparing radioautograms of the chromatograms.

The radioactive metabolites were eluted from the chromatograms with methanol. The eluates were concentrated and rechromatographed with isopropanol : acetic acid : water (4:1:1) and isopropanol : ammonia (28%) : water (8:1:1) to compare the R_fs with those reported by Wightman (84).

A number of chemicals which included IAA, tryptophan, indolealdehyde, indoleacetonitrile and tryptophol were applied in

an attempt to reverse the inhibitory effect of red light on bean plants. The applications were made (a) by injection, (b) as a soil drench, and (c) by growing the seedlings in rooting solutions to which chemicals were added.

RESULTS

Preliminary Experiments

The results obtained on the effect of light quality on the germination of three species of weed seeds are presented in table II. Of the three species studied buckwheat was not light sensitive. Its germination percentage was similar in all wavelengths. The response to different spectral bands of the other two species, stinkweed and pigweed, was striking. Germination was promoted most in the orange (540 to 605 mμ) and was poor both in the dark and the white control indicating that both these species are sensitive to light of particular wavelengths. Among the different wavelengths it was found that the germination percentage in the blue and red-far-red treatments was significantly lower than in the range green to red.

Chlorophyll and carotene determination in the normal and mutant barley (var. Montcalm) showed that the normal had about twice the amount of pigment as compared to the mutant. The normal and mutant also seemed to differ in the accumulation of pigments under the different wavelengths (table III). In case of the normal the maximum chlorophyll accumulation occurred in the red and blue with the red being more than the blue. The carotene content was greatest in blue followed by red. In the mutant however both chlorophyll and carotene accumulation was maximum in the white control. The least amount of chlorophyll and carotene content for both normal and mutant were in the deep-red and red-far-red treatments.

Table II

Per Cent Germination of Buckwheat, Pigweed and Stinkweed Seeds 8 Days After Exposure to Light of Several Spectral Bands

Figures in each column followed by different letters are significantly different at the 5% level

Color	Pigweed	Stinkweed	Buckwheat
Dark	12 d	10 e	88
Blue	4 e	10 e	100
Green	22 c	20 d	96
Yellow	52 b	40 b	96
Orange	64 a	68 a	98
Red	24 c	34 c	96
Deep-Red	10 d	8 ef	92
Red-Far-Red	4 e	4 f	88
White	10 d	8 ef	100

Mean of 4 experiments using 50 seeds per treatment

Table III

Concentration of Chlorophyll and Carotene in Leaves of Normal and Mutant Barley (var. Montcalm), Measured after Exposure to 23 Cycles of Several Spectral Bands of Light

	Normal				Mutant			
Color	Chloro. a	Chloro. b	Chloro. a + b	Carotene	Chloro. a	Chloro. b	Chloro. a + b	Carotene
Blue	0.492	0.264	0.757	0.482	0.263	0.161	0.424	0.259
Green	0.487	0.252	0.739	0.457	0.272	0.121	0.393	0.270
Yellow	0.487	0.230	0.717	0.458	0.251	0.106	0.357	0.249
Orange	0.452	0.229	0.681	0.421	0.232	0.086	0.318	0.238
Red	0.507	0.265	0.772	0.479	0.255	0.123	0.378	0.263
Deep-Red	0.443	0.226	0.669	0.447	0.222	0.101	0.323	0.233
Red-Far-Red	0.357	0.256	0.613	0.410	0.185	0.120	0.305	0.241
White	0.476	0.270	0.746	0.450	0.318	0.179	0.497	0.313

Determinations were done in duplicate and are expressed as mg/g fresh weight.

At the relatively low light intensities available in the growth cabinets it was necessary to use large seeded species which gave uniform germination. To study the effects of light quality on a monocotyledon, Zea mays (corn, var. Altagold) was selected. The seeds were germinated directly in the growth cabinets and the results obtained after 12 days of exposure to the different wavelengths are presented in table IV. Plants grown in the dark had the longest first and second internodes and coleoptiles. Red light inhibited the first internode most, and white light was most effective in inhibiting the length of the second internode and coleoptile. The primary, second and third leaves were the longest in the green cabinet and the shortest in the red-far-red cabinet. The primary leaf attained the greatest width in the deep-red and least in the red-far-red and blue. The third leaf in the red-far-red cabinet was just emerging while in all other cabinets they were relatively more developed. The dry weight of the shoots were maximum in the green whereas the dry weight of the roots were most in the deep-red cabinet. The least dry weight of both shoots and roots were in the red-far-red cabinet, being approximately half the amount compared to other light treatments. The per cent nitrogen was found to be most in the blue treated and least in the red-far-red treated plants. Surprisingly, the per cent nitrogen in the red-far-red treated plants was even lower than those of the dark control.

In the course of these preliminary experiments it was observed that the effects of light quality on morphological expression were relatively greater in dicotyledons than monocotyledons.

Table IV

Formative Effects and Nitrogen % of Corn (var. Altagold) Seedlings Exposed to 12 Cycles of Several Spectral Bands of Light

Color	Dark	Blue	Green	Yellow	Orange	Red	Deep-Red	Red-Far-Red	White
Length (cm)									
First internode	7.0	2.3	3.3	2.1	1.9	1.7	2.0	2.7	2.2
Second internode	10.1	7.7	8.7	8.7	8.1	8.5	8.5	8.0	7.5
Coleoptile	7.1	4.6	5.6	4.6	4.3	4.6	4.4	4.2	3.8
Primary leaf	7.5	7.7	8.1	8.0	7.9	7.9	7.3	6.9	7.2
Second leaf	12.2	13.9	15.0	12.4	11.7	11.1	13.9	9.7	10.6
Third leaf	3.2	4.1	8.8	5.1	2.2	2.2	6.4	0.7	1.2
Width (cm)									
Primary leaf	1.3	1.1	1.3	1.3	1.2	1.3	1.4	1.1	1.2
Dry wt (mg)									
Shoot	49	44	55	44	42	39	51	28	41
Root	12	10	12	11	12	12	16	7	9
% Nitrogen in leaves	4.5	5.8	4.1	4.7	4.9	4.4	5.4	3.5	5.4

Average of 10 plants.

Among the large-seeded dicotyledonous species tested one variety of beans called Dutch brown, obtained from Holland was found to have very high (90 - 100%) and uniform germination. It was used as the test plant for the major part of this study.

Elongation and Leaf Area Measurements

As described in methods the beans were germinated in the dark for 5 days and the seedlings were 4 to 5 cm tall when they were exposed to 7 cycles (8 hours light and 16 hours darkness) of different wavelengths. Measurements of hypocotyl, epicotyl, petiole length and leaf area are presented in table V and illustrated in figure 3 (A and B). Plants grown in the dark were etiolated and had the longest hypocotyls and epicotyls, with the shortest petioles. Of the light treated plants, those exposed to blue were tallest with the longest petioles. The plants grown in longer wavelengths had progressively shorter hypocotyls, epicotyls and petioles. This trend reached a maximum in the red light and was reversed by the deep-red and red-far-red treatments. The leaves in the dark were very small and folded having a leaf area of only 2 sq cm as opposed to the leaves in the red which had a maximum of 25 sq cm. In comparison to the red treatment leaves from the blue and red-far-red treated plants were significantly smaller having a leaf area of 16 and 18 sq cm respectively.

Dry Weight Determinations

The dry weights of the bean plants exposed to 7 cycles of different lights are given in table VI. The determinations were done separately for the shoots, leaves, cotyledons and roots. Dark grown

Table V

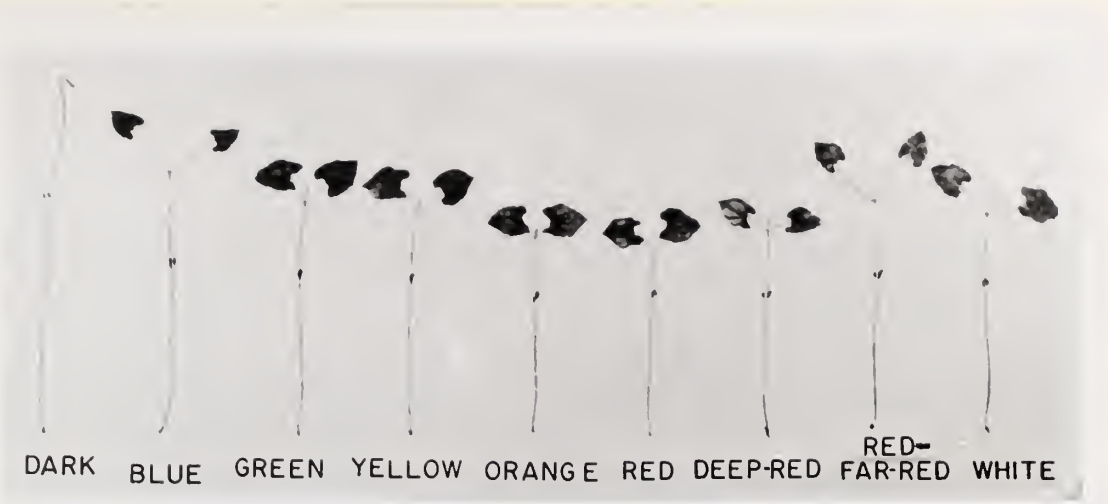
Effect of Several Spectral Bands of Light on Elongation and Leaf Area of Bean Plants Irradiated for 7 Cycles

Figures in each column followed by different letters are significantly different at the 5% level

	Length (cm)			Area (sq cm)
Color	Hypocotyl	Epicotyl	Petiole	Leaf
Dark	25.4 a	14.6 a	1.1 f	2 d
Blue	21.6 b	13.3 b	7.3 a	16 c
Green	18.5 d	10.0 d	3.5 de	22 b
Yellow	18.2 de	9.3 e	4.0 cd	24 ab
Orange	17.5 ef	8.6 f	3.5 de	24 ab
Red	16.9 f	8.0 g	3.1 e	25 a
Deep-Red	18.2 de	10.1 d	4.7 b	23 ab
Red-Far-Red	20.0 c	11.5 c	7.0 a	18 c
White	18.5 d	10.4 d	4.5 bc	23 ab

Results are an average of 6 experiments using 13 plants per treatment.

A



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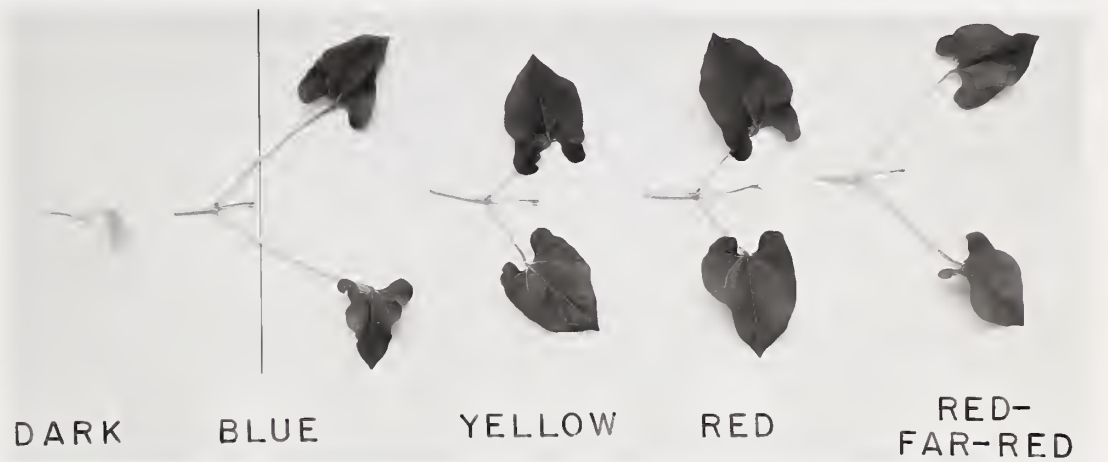


FIG. 3. Bean plants irradiated for 7 cycles with several spectral bands of light. A, showing hypocotyl, epicotyl, petiole elongation and leaf area; B, enlarged portion of a few treatments to illustrate petiole length and leaf area.

Table VI

Dry Weight of Bean Plants Irradiated
with 7 Cycles of Several Spectral Bands of Light

Figures in each column followed by different letters are significantly different at the 5% level

	Dry wt/plant in mg				
Color	Shoot	Leaves	Cotyledons	Roots	Total
Dark	152 a	17 f	73 a	23 c	267 a
Blue	116 b	44 e	67 bc	28 abc	255 ab
Green	88 e	62 bc	66 bc	27 abc	243 b
Yellow	87 e	62 bc	69 abc	32 a	250 b
Orange	86 e	64 ab	63 cd	32 a	245 b
Red	86 e	68 a	60 d	30 ab	246 b
Deep-Red	90 de	58 cd	65 cd	31 a	245 b
Red-Far-Red	104 c	48 e	63 cd	25 bc	240 b
White	95 d	54 d	71 ab	29 ab	250 b

Mean of 2 experiments using 13 plants per treatment.

plants had the significantly highest shoot and lowest leaf weight. Blue and red-far-red treated plants had a greater shoot and lower leaf weight than plants from other light treatments. Leaves from the red treated plants had significantly greater dry weight than all except orange light treatments. The dry weight of the cotyledons from the dark grown plants were similar to those from the white and yellow but significantly greater than all other treatments. Root weight from plants in the dark, was not different from the blue, green, and red-far-red but was significantly lower than in the yellow to deep-red range. The total dry weight of the dark grown plants was significantly higher than all other treatments except the blue.

An experiment was done to determine whether an inductive effect of 1 cycle was enough to affect elongation. Plants were exposed to 1 light cycle and then put in the dark for 6 days. Although the differences were not as pronounced after 1 light cycle as after 7, it is evident from figure 4 that the same trend was obtained, with the plants in the red being the shortest.

The average lengths of individually pre-marked hypocotyl segments and epicotyl elongation data are presented in table VII. There was practically no elongation in the first five segments in any wavelength. In the sixth segment the blue has an increase of 0.4 cm. In all other wavelengths there was an increase of about 0.1 cm. In the seventh segment the blue increased by 1.2 cm and the increase in all other wavelengths was less than 0.7 cm. Plants in the blue treatment also had the longest eighth segment with the red being the shortest. The ninth and the tenth segments were the regions of

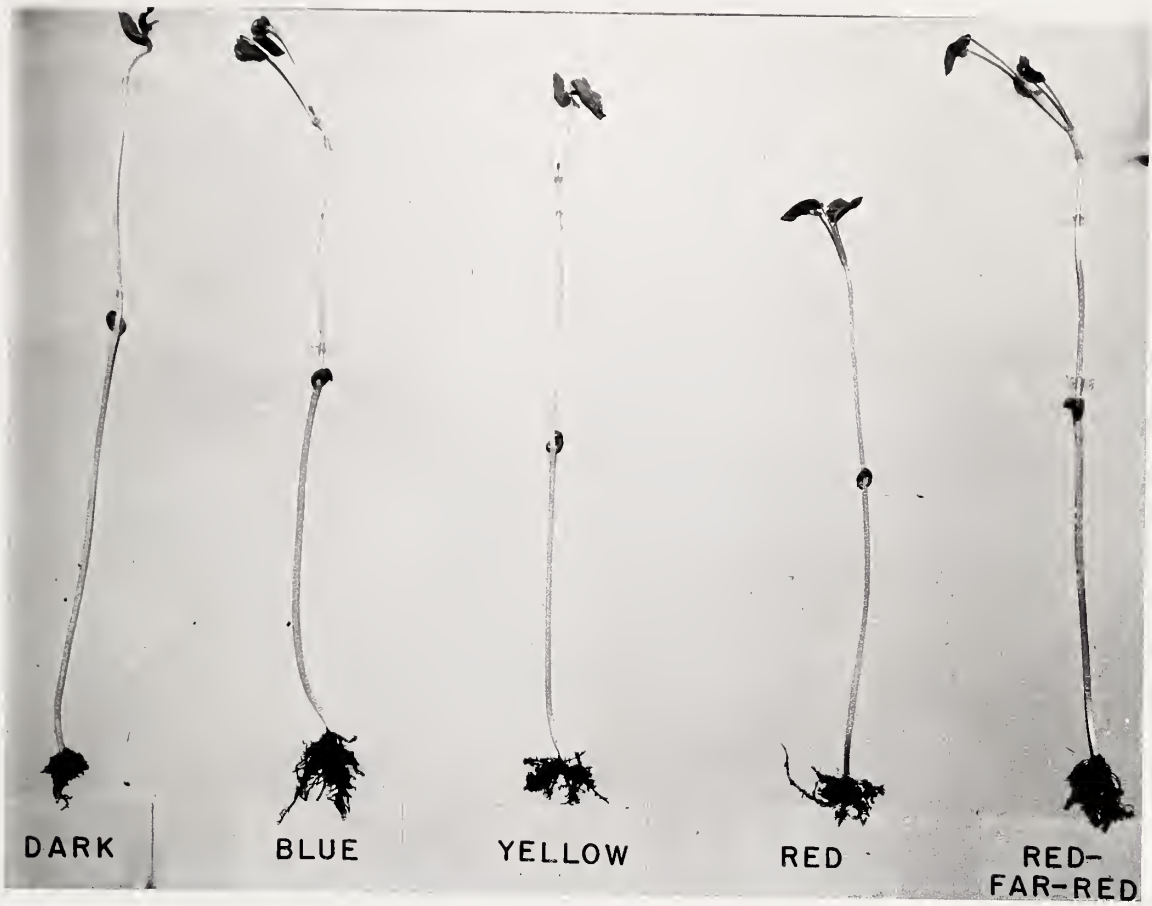


FIG. 4. Bean plants irradiated with 1 cycle of several spectral bands of light and then grown in the dark for 6 days.

Table VII

Effect of Several Spectral Bands of Light on the Elongation of Epicotyls and Marked Segments of Bean Hypocotyls

Color	Length of individual segments of hypocotyl							Hypo-cotyl	Epi-cotyl	Total height
	4	5	6	7	8	9	10			
Dark	1.2	1.1	1.1	1.8	2.6	4.1	13.1	28.0	8.8	36.8
Blue	1.1	1.1	1.4	2.2	3.5	8.2	4.2	24.7	8.5	33.2
Green	1.0	1.0	1.1	1.7	2.5	5.0	4.8	20.1	7.6	27.7
Yellow	1.0	1.0	1.1	1.5	2.1	6.1	4.6	20.4	7.5	27.9
Orange	1.0	1.0	1.1	1.3	2.0	5.8	3.7	18.9	6.1	25.0
Red	1.0	1.0	1.0	1.2	2.0	5.5	2.5	17.2	5.5	22.7
Deep-Red	1.0	1.1	1.1	1.4	2.1	7.0	4.5	21.2	7.8	29.0
Red-Far-Red	1.0	1.1	1.2	1.5	2.3	7.1	4.6	21.8	8.1	29.9
White	1.0	1.0	1.1	1.3	2.1	4.6	4.3	18.4	6.5	24.9

Average of 8 plants measured in cm. Segments 1, 2 and 3 were all 1 cm.

greatest elongation in all wavelengths with the blue (fig 5), red-far-red and deep-red having the maximum elongation in that order. An interesting observation here is that the dark control plants had the least elongation in the ninth segment but the most elongation in the tenth segment, having about a 3-fold increase of elongation over most of the other wavelengths. For this segment the increase of the dark compared to the red was more than 5-fold. The lengths of the hypocotyls, epicotyls and total height of plants followed the same pattern as the earlier results on elongation.

Root Formation

The number of adventitious roots formed on the hypocotyls of excised bean seedlings after various treatments of different light quality are presented in table VIII and illustrated in figure 6 (A and B). In all the experiments the largest number of roots were formed in the red treated plants. When the excised seedlings were grown continuously for 5 cycles in the different growth cabinets, both blue and red-far-red treated seedlings formed the least number of roots. The blue treated seedlings had less than half the number of roots as the red treatment. In order to determine whether an inductive effect would be enough to bring about these differences, the excised seedlings were exposed to 1 cycle of different wavelengths of light and then one set was put in darkness and the other set in the white cabinet. Following five days in the dark it was found that the cuttings from the red pre-treated plants had the largest number (67) of roots. The red-far-red pre-treated cuttings were the most inhibited having only 4 roots. However, following 5 days in the

FIG. 5. Seven day old bean seedlings with the hypocotyl marked into ten segments and then exposed to 3 cycles of blue light.

Table VIII

Effect of Light of Several Spectral Bands on
Number of Adventitious Roots Formed in Excised Bean Seedlings

Color	Dark	Blue	Yellow	Red	Red-Far-Red
5 cycles	53	24	51	59	37
1 cycle + 5 days darkness	30	22	59	67	4
1 cycle + 5 days white cabinet	59	49	53	60	55

Mean of 2 experiments using 6 plants per treatment.

A

B

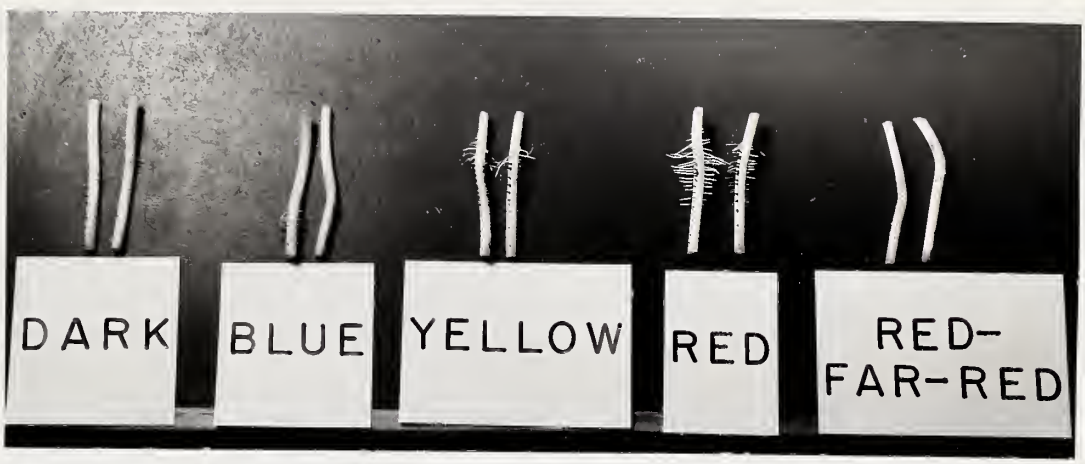


FIG. 6. Roots formed on the hypocotyls of excised bean seedlings exposed to several spectral bands of light, A, for 5 cycles; B, for 1 cycle and then grown in the dark. Leaves and stem tips were removed when the pictures were taken.

white cabinet the inductive or inhibitory effects of the different wavelengths was essentially overcome. Although this study involved only a root count and measurements of root length were not made, it can be observed (fig 6, A and B) that the red treated plants not only had the largest number of roots but they also had the longest roots. The roots in the red treatment were about 2 cm long whereas the roots formed in all other wavelengths did not exceed 1 cm.

Pigment Determinations

The total chlorophyll and carotene content in the primary leaves of bean plants exposed to the different wavelengths of light are shown in figure 7. Maximum synthesis of chlorophyll (1.37 mg/g fresh weight) occurred in the red followed by blue with a depression in the green yellow range. The leaves of the plants treated with red-far-red light were chlorotic and the chlorophyll content was about 60% of that in plants from the red cabinet. Carotene content followed the same trend as chlorophyll with a maximum of 0.75 mg/g fresh weight in the red cabinet and a minimum of 0.41 mg/g fresh weight in the red-far-red cabinet.

Nitrogen and Amino Acid Determination

Nitrogen estimations were done on the particulate, acid-insoluble and soluble fractions from the leaves of bean plants exposed to 5 cycles of light (table IX). The acid-insoluble fraction had relatively slight differences in the amount of nitrogen in the different treatments. However, there were some striking differences in the acid-soluble fraction with the dark, blue, and white treatments

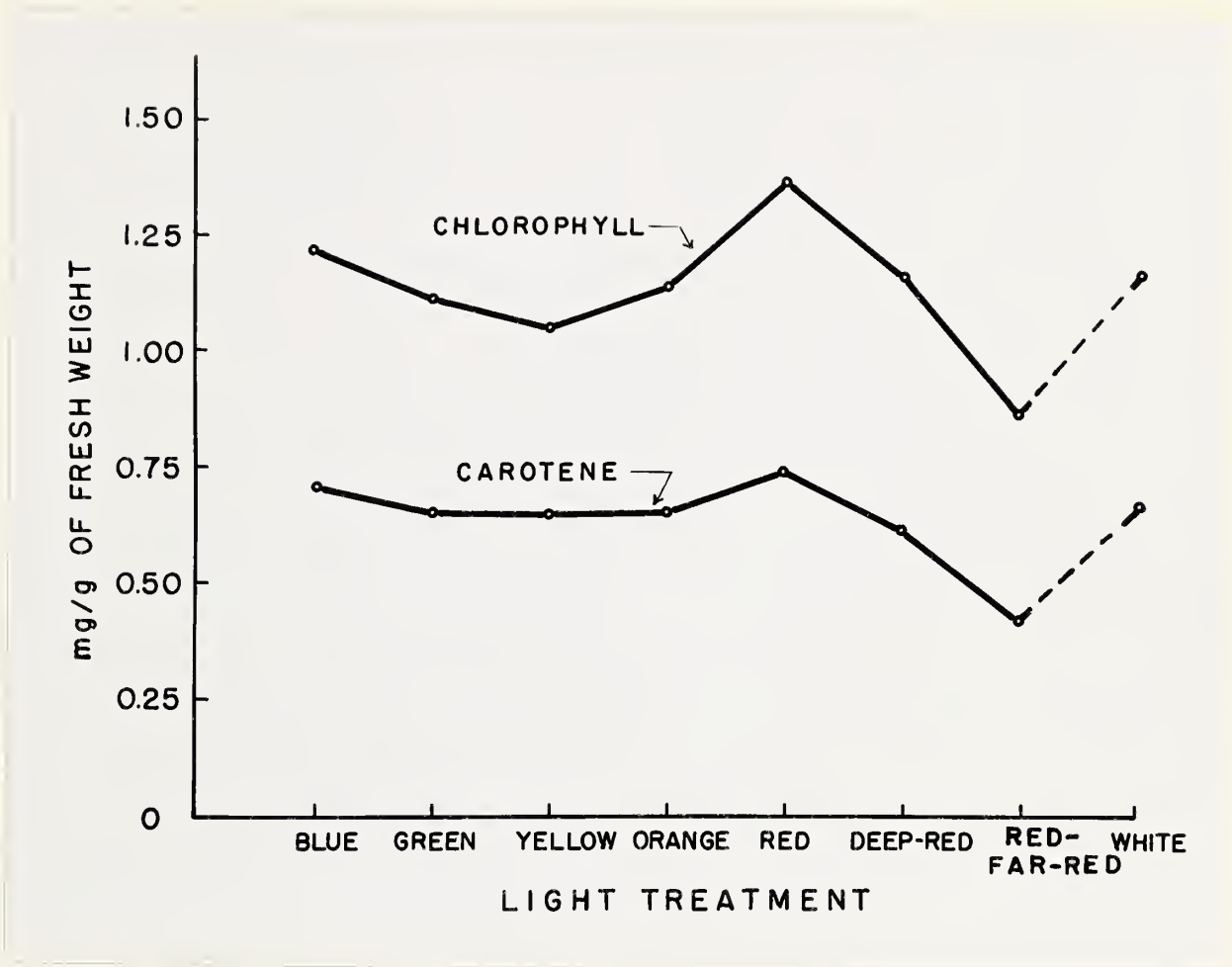


FIG. 7. Total chlorophyll and carotene content in primary leaves of beans after exposure to 7 cycles of light of different spectral bands.

Table IX

Distribution of Nitrogen in Leaf Fractions of Bean Plants Exposed to 5 Cycles of Several Spectral Bands of Light

	mg of Nitrogen/gm dry weight in			
Color	Particulate	Acid-insoluble	Acid-soluble	Total
Dark	41.1	19.3	16.9	77.3
Blue	43.0	21.2	16.9	81.1
Green	47.1	22.8	6.9	76.8
Yellow	43.3	22.9	9.1	75.3
Orange	44.7	20.9	8.3	73.9
Red	41.7	21.0	10.8	73.5
Deep-Red	40.0	22.6	13.7	76.3
Red-Far-Red	49.8	22.2	8.4	80.4
White	39.6	20.1	17.8	77.5

Determinations in duplicate.

having more than twice the amount of nitrogen as the green treatment. In general there was an inverse relation between the amount of nitrogen in the particulate fraction with the amount in the acid-soluble. The total nitrogens were therefore similar.

The acid-soluble fraction of the dark, blue, green and deep-red treatments were analyzed for their amino acid content (table X). The leaves of the dark control plants had more of all amino acids except serine, valine and isoleucine. Leaves from the green treatment had the least amount of almost all the amino acids and had the highest ammonia. A comparison of the amino acid content from leaves exposed to blue and deep-red light showed that there was an appreciable difference in the following amino acids. Blue had more histidine, aspartic acid, threonine, and serine. When the total free amino acid concentrations were compared it was found that the dark control had the most. Blue had a greater concentration than the red with the green being the least.

Measurement of Endogenous IAA

Since the plant tissue which included the cotyledons had a relatively high IAA content, 2 plants (approximately 1.5 g fresh wt) were sufficient to detect IAA both by chromogenic reactions as well as by the spectrophotometric method (19). The absorption curve (fig 8, B) obtained from the eluate of the region R_f 0.32 to 0.35 was identical in its characteristics with that of synthetic IAA (fig 8, A). When the same procedure was followed with a greater

Table X

Free Amino Acid Concentrations in the Acid Soluble Fraction of Leaves of Bean Plants Exposed to 5 Cycles of Light

Micromoles of amino acids per gram fresh wt

Amino Acid	Dark	Blue	Green	Deep-Red
Lysine	0.51	0.26	0.21	0.26
Histidine	4.26	3.52	2.28	2.80
Ammonia	4.00	3.58	5.13	4.20
Arginine	1.13	0.56	0.26	0.43
Aspartic Acid	2.55	2.39	0.68	1.73
Threonine	2.21	1.57	1.07	0.99
Serine	8.90	10.82	5.56	8.72
Glutamic Acid	5.39	1.53	1.23	1.58
Proline	-	-	-	Trace
Glycine	0.87	0.86	0.22	0.78
Alanine	1.47	0.67	0.34	0.61
Half Cystine	-	-	-	-
Valine	0.72	0.70	0.65	0.81
Methionine	-	-	-	-
Isoleucine	0.25	0.38	0.41	0.44
Leucine	0.46	0.28	0.22	0.32
Tyrosine	0.43	0.31	0.12	0.21
Phenylalanine	0.95	0.15	0.10	0.16
Total	34.10	27.60	18.48	24.04

quantity (3 g) of fresh material resolution was unsatisfactory and contamination of the eluate occurred as evidenced by the second absorption peak at 273 mμ (fig 8, D). This was probably due to interference of an unknown compound obtained from R_f 0.29 - 0.32 (fig 8, C). Later it was found that by using 7% instead of 28% ammonia in the solvent isopropanol : ammonia : water (8:1:1), the interfering substances such as chlorophyll or other pigments, either moved with the solvent front or stayed close to the line of application (20). The unknown compound which interfered with the resolution also separated from IAA with this solvent. Therefore these substances did not interfere with the resolution of IAA, and fractionation of the crude extract prior to chromatography was not necessary. The R_f values of synthetic and endogenous IAA in the isopropanol : ammonia : water (8:1:1) solvent systems using 7% (R_f 0.49) and 28% (R_f 0.34) ammonia were the same. Their chromogenic reactions with the Salkowski (pink), Ehrlich (blue) and DMCA (purple) reagents were identical.

The absorption at 280 mμ of methanol solutions of synthetic IAA obeys Beer's law over at least the range of 1 - 30 μg/ml (fig 9, A). When known concentrations of synthetic IAA were added to the crude extract and the same isolation procedure followed, the absorption values of the eluate at 280 mμ were additive (fig 9, B). The sensitivity of spectrophotometric measurements was determined to be 0.2 μg/ml at a concentration of 9 - 10 μg/ml and a mean error of 1.1 per cent (of the mean concentration) was established using 10 replications of 1.5 g of fresh material.

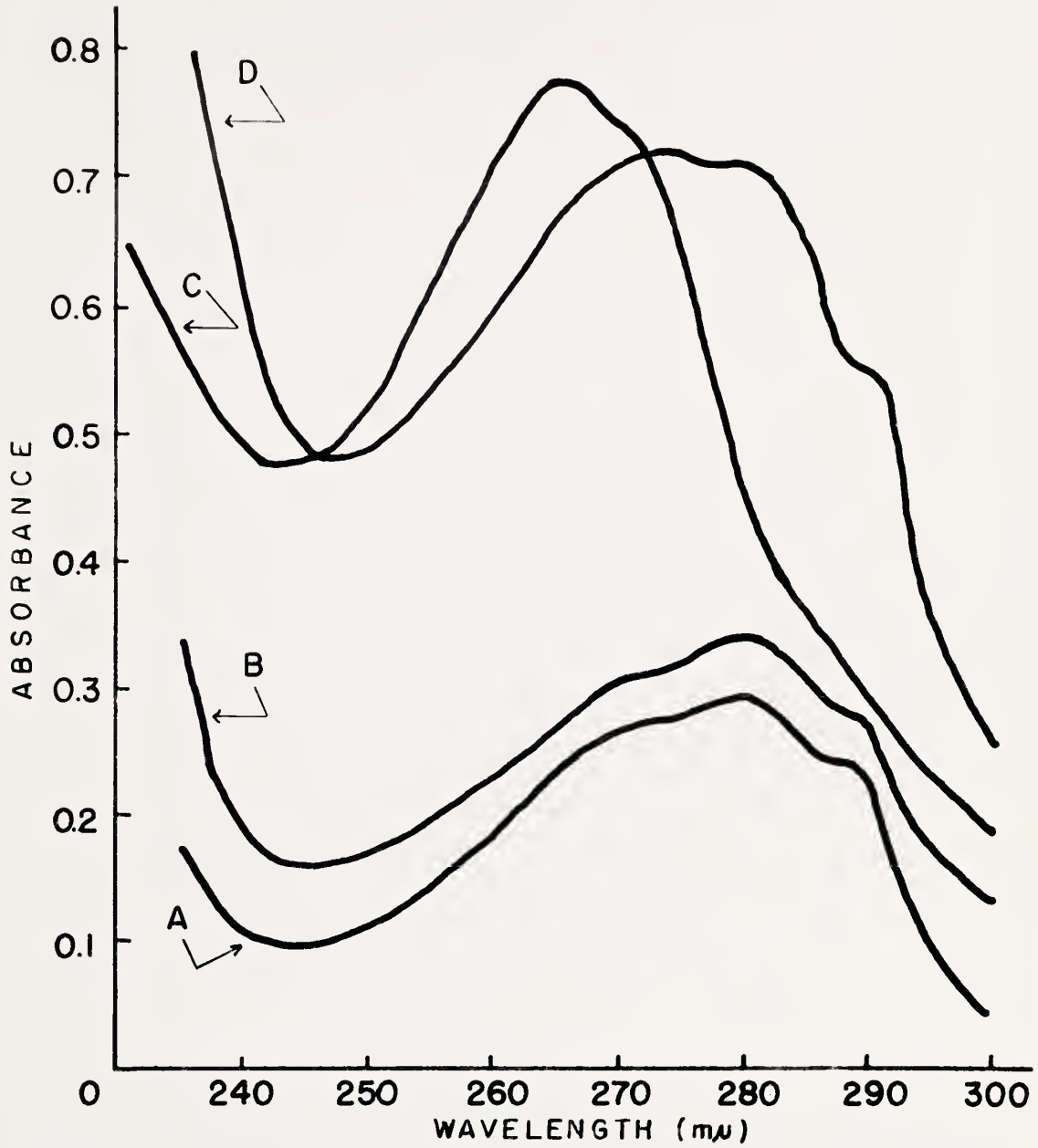


FIG. 8. Ultra-violet absorption curves. A, synthetic IAA (10 μg/ml); B, IAA (R_f 0.32 - 0.35) from 1.5 g fresh weight; C, unknown compound (R_f 0.29 - 0.32) from 1.5 g fresh weight; D, contaminated IAA (R_f 0.32 - 0.35) from 3 g fresh weight.

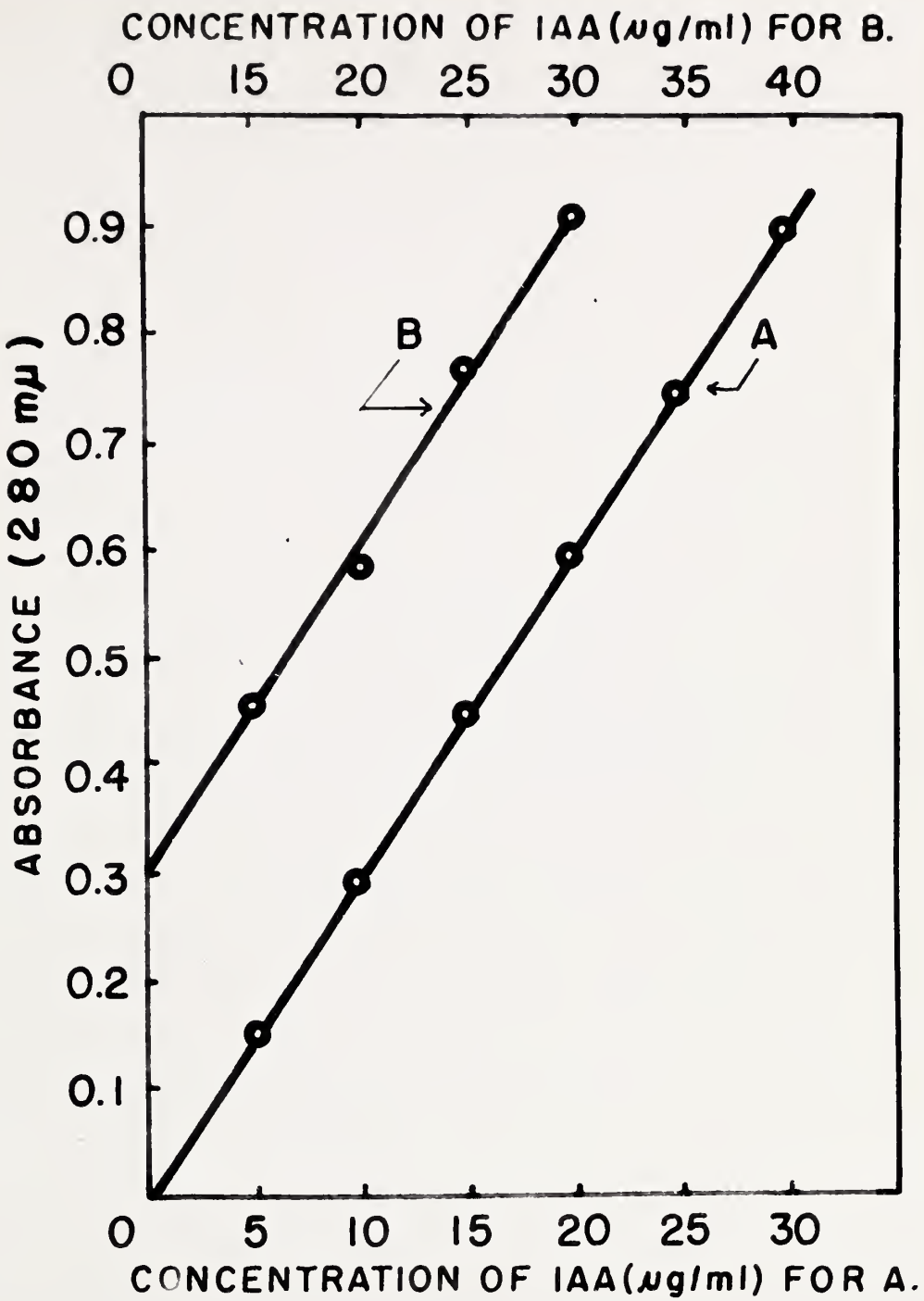


FIG. 9. A, Relationship between concentration and absorbance of synthetic IAA in methanol; B, IAA extracted from 1.5 g fresh material + 0, 5, 10, 15, 20 µg/ml synthetic IAA.

A standard curve for IAA based on the first internode test using Vicar oats, is given in figure 10. The IAA content in ug/plant after 1 cycle of different light treatments were determined for 4 experiments using 13 plants per treatment by both the uv method and the bioassay. The values obtained are shown in the following tabulation:

	Color				
Method	Dark	Blue	Yellow	Red	Red-Far-Red
UV	12.91	11.66	10.25	8.83	10.58
First internode	13.21	11.92	10.42	9.04	10.96

The concentrations of IAA obtained by the two methods agreed closely. The correlation coefficient was + 0.84.

An analysis of variance for these data gave the following values for the components of variance.

Source of Variation	DF	Variance
Method	1	1.0
Treatments	4	19.5**
Treatment x Method	4	0.0
Error	27	0.4

Although the differences between treatments were highly statistically significant, the methods and the interaction, treatment x method were not significant.

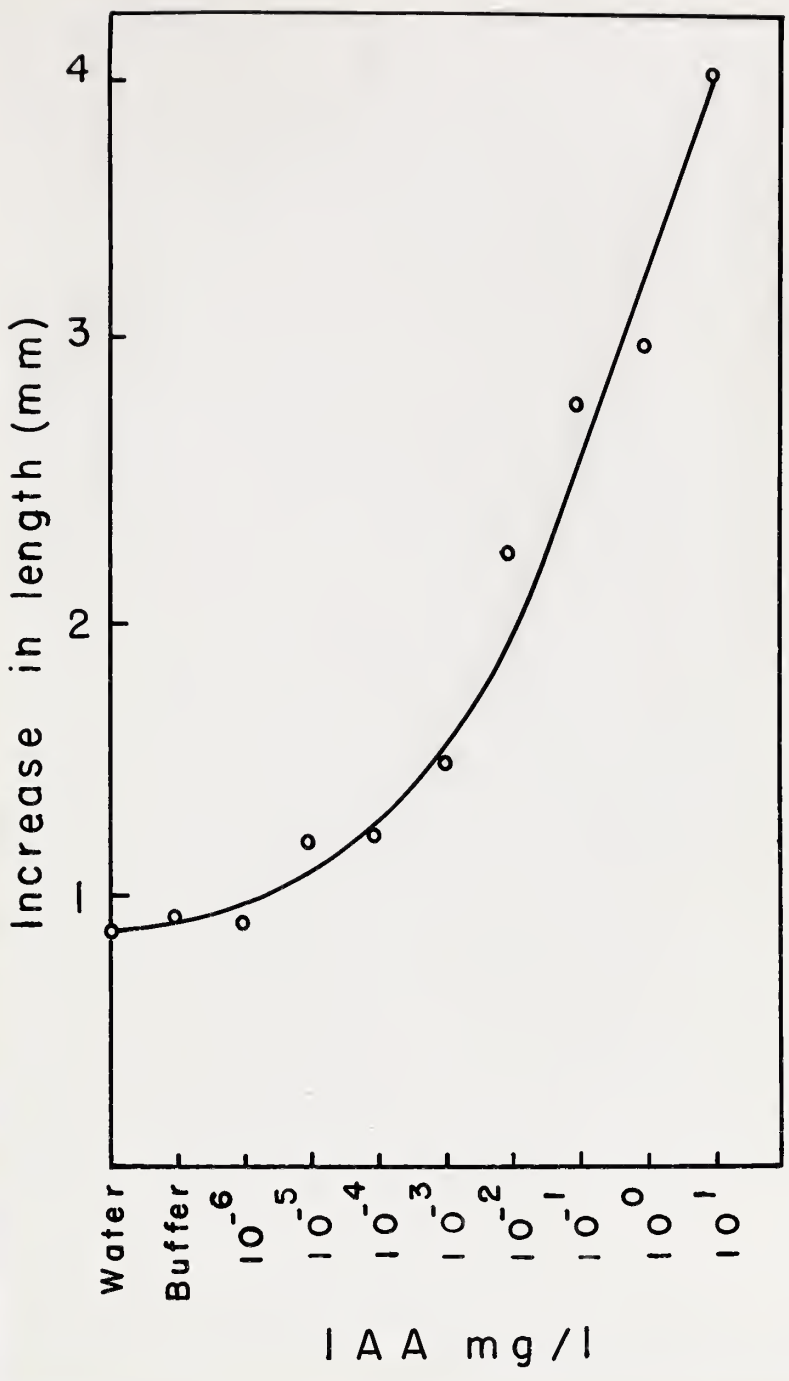


FIG. 10. Straight growth response of oat first internode sections in serial dilutions of IAA.

Employing the uv method it was found that plants grown in the dark for 5 days contained 12.5 µg IAA/plant. The levels of IAA after 1 and 7 cycles as determined by this method are given in table XI. As can be seen all the treatments after both the 1 and 7 cycle exposure were significantly different from each other except yellow and red-far-red. Plants kept in the dark had the most IAA and those treated with red light had the least.

The levels of IAA after the 1 and 7 cycle exposure to different light treatments in general followed the same trend but their difference was significant. All treatments had a significantly greater increase than red.

Since the dark grown plants before treatment contained 12.5 µg IAA and the dark control plants after 1 cycle had only 12.9 µg there was no appreciable production of IAA. All the light treated plants had a lower concentration than the initial value indicating that there may have been either destruction or binding of IAA at this stage. After 7 cycles the dark control plants had an increase of 2.2 µg IAA whereas the increase in the red was significantly less being only 0.74 µg.

When the values of IAA concentrations obtained after 1 and 7 light cycles are compared to the height of plants a direct relationship can be seen (fig 11).

When solutions of aqueous IAA (10.3 mg/l) were exposed to different light treatments, there was no evidence of photodestruction. However, in the presence of bean sections there was a loss in detectable IAA of about 2.5 mg/l in all the treatments.

Table XI

Concentration of IAA in Bean Plants after Irradiation for 1 and 7 Cycles with Light of Several Spectral Bands

Figures in each column followed by different letters are significantly different at the 5% level

Color	µg IAA/plant		Increase (7-1)
	1 Cycle	7 Cycles	
Dark	12.91 a	15.11 a	2.22 a
Blue	11.66 b	13.71 b	2.1 a
Yellow	10.25 c	11.75 c	1.55 b
Red	8.83 d	9.54 d	0.74 c
Red-Far-Red	10.58 c	12.47 c	1.97 ab

Mean of 4 experiments using 13 plants per treatment.

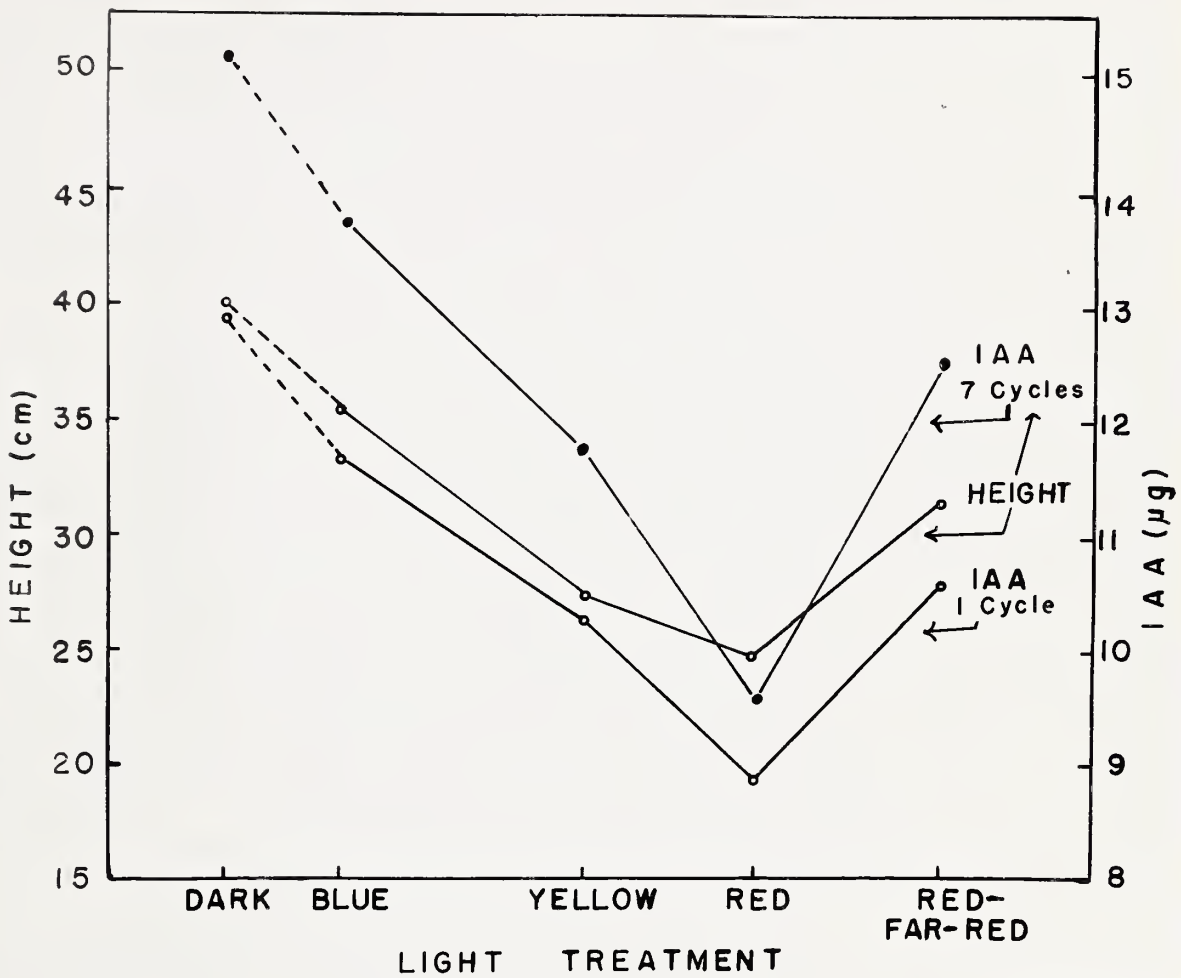


FIG. 11. Relationship between plant height (hypocotyl plus epicotyl) after 7 light cycles and IAA content per plant after 1 and 7 cycles.

Metabolic Studies with IAA

The recovery of IAA from bean seedlings injected with 100 µg IAA followed by exposure to 1 cycle of light is given in table XII. The greatest recovery of 56% was obtained from the dark control plants. There was considerably lower recovery from plants exposed to light. Blue light gave the highest recovery, 47%, yellow and red the least, 40%.

Radioactive IAA

A typical radioautogram of a bean plant injected with 0.2 µc of radioactive IAA followed by 1 cycle of treatment is presented in figure 12. It is evident that IAA from the cotyledons is translocated both basipetally as well as acropetally. Radioautograms developed from plants which were quick frozen either in the deep freeze (-30 C) or by dipping in isopentane-dry ice (-80 C) showed the same distribution of label. There was no visible difference of translocation in plants exposed to light of different spectral bands.

The relative activity in the three fractions designated I - free IAA, II - bound IAA, III - strongly bound IAA are presented in table XIII. Dark treated plants yielded 81% of the activity in fraction I whereas red treated plants had only 41% of the total activity in this fraction. In fraction II the yields were reversed with red treated plants having 31% of the activity as compared to the dark which had 11%.

It is evident from the results that very little activity was recovered in fraction III. The total activity recovered from the dark

Table XII

Recovery of IAA from Plants which were Injected with 100 µg IAA/plant and then Exposed to 1 Cycle of Different Spectral Bands of Light

Color	IAA µg/plant		Recovery %
	Total Recovered	Endogenous*	
Dark	68.8	12.9	56
Blue	58.3	11.7	47
Yellow	50.2	10.3	40
Red	48.9	8.9	40
Red-Far-Red	53.2	10.6	43

* Previously determined.

Mean of 4 experiments using 13 plants per treatment. SE did not exceed ± 0.32.

FIG. 12. Radioautogram of a bean seedling showing translocation of radioactive IAA. The IAA was administered to the cotyledons and the seedlings were exposed to 1 cycle of light.

Table XIII

Effect of Light of Several Spectral Bands on the Recovery of Radioactive IAA Injected into the Cotyledons of Bean Seedlings

The cpm are expressed as a per cent of the total administered per plant.

Color	Fraction I Free IAA	Fraction II Bound IAA	Fraction III Strongly Bound IAA	Total Recovered
Dark	81	11	2	94
Blue	75	12	4	91
Yellow	60	17	5	82
Red	41	31	7	79
Red-Far-Red	66	13	5	84

Mean of 3 experiments.

plants was 94%. In the red treated plants in spite of the 3 extractions only 79% of the activity could be recovered, the remaining 21% could not be accounted for.

To determine the relative activity of the individual radioactive metabolites in each fraction, and to determine how much of the total activity in each fraction was due to radioactive IAA, the blue and red light treatments were selected for chromatographic study. Aliquots from each fraction of these were chromatographed in isopropanol : ammonia 7% : H_2O (8:1:1) and scanned with a 4π chromatogram scanner. It was found that in fractions I and II, besides IAA there were two additional radioactive metabolites having similar R_fs in both fractions. One of these had an R_f of 0.02 and is called X and the other having an R_f (0.84) is designated as Y. The metabolites contributing to the low activity in fraction III could not be detected by the scanner.

Radioautograms were prepared of the developed chromatograms containing the two fractions as well as a marker with radioactive IAA. The position of the recovered IAA corresponded to that of the marker. Figure 13 shows the similarity between IAA in one of the fractions and synthetic IAA as well as the position of the metabolites X and Y.

The following tabulation presents the relative activity in each metabolite in fractions I and II.

		Metabolite (%)		
		IAA	X	Y
Blue	I	59	26	15
	II	52	39	9
Red	I	41	28	31
	II	45	36	19

More IAA was recovered from the blue treatment in fraction I than the red. There was very little difference in the amount of X. However there was almost twice the amount of metabolite Y in the red as in the blue treatment. The relative activity of IAA and the two metabolites X and Y in fraction II followed a similar pattern as in fraction I. When the activity due to IAA was totalled in the two fractions it was found that in the blue IAA contributed 55% of the activity whereas in the red it contributed 43%.

The metabolites were eluted and rechromatogrammed in two solvent systems. On this basis one of the metabolites (Y) has been tentatively identified as indolealdehyde, since its R_f compares closely with that of the synthetic compound and with the values given by Wightman (84) for this compound in different solvent systems. Although the R_f (0.02) of X is similar to indoleacetylaspartic acid, its identity has not been established since its R_f has not been compared with the synthetic compound.

The attempt to reverse the inhibitory effect of red light on elongation of bean plants was not successful. A number of chemicals which included tryptophan, indolealdehyde, indoleacetonitrile and

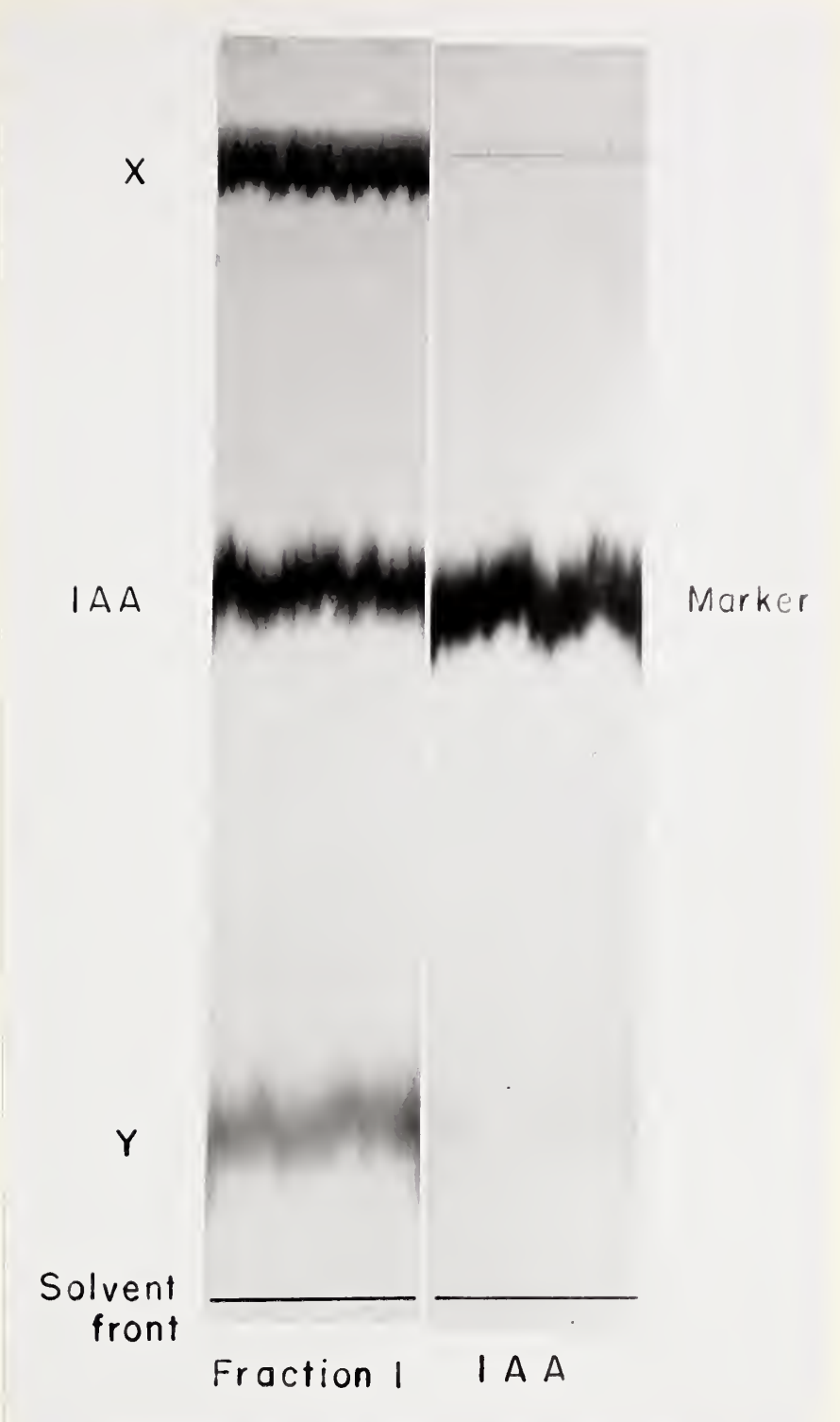


FIG. 13. Radioautographs of chromatograms developed in isopropanol : ammonia 7% : H_2O (8:1:1), A, Fraction I; B, IAA marker.

tryptophol failed to reverse the inhibitory effect of red light upon elongation. Although a complete reversal was not obtained when IAA was applied, it was observed from a number of experiments that the hypocotyl and first internode lengths increased by about 1 cm over the control.

DISCUSSION

The morphogenetic responses of plants to light of different spectral bands is dependent upon the intensity (52) and plant materials (72) used. In this study the energy obtained in the various growth cabinets was 2900 ergs/cm^2/sec which is considered to be a relatively low intensity for photosynthesis. Although the light intensities in the growth cabinets were adjusted to provide uniform energy, the quantum fluxes in the cabinets were not the same, the value at the red end of the spectrum being almost twice that at the blue. Since standardization was not on the basis of quantum flux, this might also contribute to differences in rates of photosynthesis in the various treatments.

Another complicating factor in assessing the role of light was the long duration of the treatments, thereby implicating factors other than inductive effects. There was considerable overlapping of wavelengths in the various growth cabinets. Overlapping of far-red (wavelengths above 730 mμ) into the red region of approximately 10, 30 and 50% of peak transmittance occurred respectively in the red, deep-red and red-far-red cabinets. In view of what is known about the red-far-red reversal phenomenon this overlapping could considerably modify the red effect. Some of these limiting and complicating factors must be borne in mind in assessing the results of this study.

The spectrophotometric method developed by Fletcher and Zalik (19) and employed in this study, was found to be satisfactory in the assay of IAA in small samples of plant tissue. This method involves

very simple extraction, chromatography and spectrophotometric measurement. Although it is less sensitive than the first internode bioassay it has the advantages of greater simplicity, precision, specificity and convenience. The concentrations of IAA obtained by using the spectrophotometric and the bioassay methods agreed closely. An analysis of variance showed that there was no significant difference in the results obtained by these two methods.

The IAA content in the bean seedlings including the cotyledons was found to be relatively high. Preliminary studies indicated that most of the endogenous IAA was in the cotyledons. When radioactive IAA was applied to them there was translocation both basipetally and acropetally (fig 12). This suggests that in the seedling stage the endogenous IAA in the cotyledons could be translocated in a similar manner.

It was demonstrated by Van Overbeek (58) in 1936 that light in the yellow to red region lowers the level of diffusible auxin in Avena coleoptiles. A lowering of IAA levels in coleoptiles by both red and far-red light has been reported by Blaauw-Jansen (9). In a recent study Briggs (13), using corn coleoptiles excised from red treated seedlings, found that a two hour exposure reduced the IAA content to about half that in the dark. In this study it was found that after 1 light cycle the concentration of IAA in the red treated plants was reduced to about 68% of the amount in the dark control. Although the greatest reduction of IAA was in the red treated plants, other treatments, such as blue, yellow and red-far-red, also caused a significant reduction in IAA levels when compared to the dark treatment.

It was found that the IAA content in the dark control plants had increased very little in 24 hours over the initial concentration of 12.5 µg/plant in 5 day old seedlings. This suggests that Δ_{net} production of IAA was not an important factor at this stage. Considering that all the light treated plants had a lower level of IAA than the initial concentrations, there must have been either photodestruction, conversion or binding of IAA.

The IAA concentrations after 7 cycles showed a similar trend to that found after 1 cycle, with dark having the most IAA and red the least (table XI). In the dark and blue treatments the IAA concentrations were higher than the initial amounts, indicating that production or release of IAA may be involved. In all the light treatments there was more IAA present after 7 cycles than was present after 1 cycle, with the increase in the red being significantly less than all other treatments. These results suggest that the effects of light on IAA levels may involve photodestruction, binding or production, making this problem a complex one. Leopold (45) has referred to the complexities of the role of light in IAA metabolism as a paradox, for light may be involved in the formation as well as the destruction of IAA.

The IAA concentrations determined after 1 and 7 light cycles showed a close relationship with the growth pattern after 7 cycles. This finding suggests that one of the mechanisms by which light of different wavelengths controls growth is through the regulation of endogenous levels of IAA. Meijer (52) found that an application of indole compounds, particularly tryptophol, produced elongation of plants inhibited by red light. From this indirect evidence he postulated

that IAA is involved in the effects of light quality on plant elongation. A number of chemicals, including tryptophol, did not reverse the inhibitory effect of red light on plants in our study. However, an application of IAA increased the lengths of the hypocotyls and first internode by about 1 cm over those of the control plants.

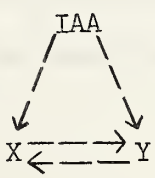
Although there was a strong relationship between the levels of free IAA and plant growth it cannot be stated unequivocally that IAA was the sole regulator of elongation. If it was the only determining agent an addition of exogenous IAA to the inhibited plants should have caused a complete reversal of the red inhibition. The inability to bring about such a reversal by the application of exogenous IAA could be due to one or more of the following: the supplied IAA may (a) have been destroyed (b) not have reached the locale of action (c) not have been converted into an essential complex to become effective (d) not have been able to overcome the effect of an inhibitor possibly produced by red light and (e) might not have interacted with other compounds such as gibberellin to become effective.

Lockhart (49, 50) interpreted his results to mean that red-far-red radiation influenced stem growth through an effect on the level of endogenous gibberellin. In a recent study, however, Roesel and Haber (63) have concluded from their work with wheat that red, blue, or white light does not regulate elongation of dark grown plants by regulating endogenous gibberellin levels.

In studies using rice coleoptiles Kefford (42) found that red grown coleoptiles were auxin and gibberellin deficient when

compared to far-red grown coleoptiles. His results supported hypotheses requiring an interaction between auxin and gibberellin in the control of coleoptile elongation, but did not support independent roles for the two in cell enlargement. Considering that the results of the present study indicate that the elongation of plants is related to endogenous IAA levels whereas the studies of Lockhart showed a relationship with gibberellin, the hypothesis of Kefford that elongation is controlled by an interaction between auxin and gibberellin is feasible. But until further evidence on the mode of action of auxin and gibberellin is available the possibility that these two compounds may have independent effects on cell enlargement cannot be ruled out.

The recovery of applied IAA from intact plants varied with the light treatments (table XII). When radioactive IAA was administered it was found that in the first fraction, which is assumed to be free IAA, most (81%) of the activity was recovered from the dark control plants, with least (41%) being recovered from the red. These results indicate that IAA metabolism was affected differently by light of different wavelengths (table XIII). Chromatographic studies detected two labelled metabolites, X and Y, in addition to the labelled IAA (fig 13). The IAA might have been converted directly or indirectly to these two compounds as illustrated.



An examination of the tabulation on page 62 indicates that there was generally more of metabolite X than Y. This compound had a similar R_f to that of indoleacetylaspartic acid. The formation of this conjugation compound in plant tissue supplied with exogenous IAA has been reported by a number of workers (1, 18, 84). Comparisons of the relative concentrations of this compound showed very little difference between that found in the blue and red light treatments.

Galston (25) states in his review that although the products of photodegradation of IAA are not completely known the appearance of indolealdehyde has been demonstrated in the riboflavin-sensitized system. Ray (62) has reviewed the work on the formation of indolealdehyde as a product of photooxidation and as a possible product of enzymatic oxidation. In the present study, metabolite Y has been tentatively identified as indolealdehyde. In both fractions I and II this compound has approximately twice the concentration in the red treatment as in the blue. It can therefore be speculated that red light stimulates the photooxidation of IAA to indolealdehyde much more readily than blue. This may in part be an explanation for the lower levels of endogenous IAA in red light treated plants. About 21% of the activity from red treated plants could not be accounted for. This may be due either to inability of total extraction by the 3 methods used, or loss during extraction, especially by acid hydrolysis which was used for obtaining fraction III, or to metabolism resulting in loss of the labelled carbon in gaseous form.

Determination of the dry weight of plants irradiated with different wavelengths gave some surprising results (table VI). The total dry weight of plants of the dark control was significantly higher than those in all other treatments except blue. Also, the weight of the shoots of the dark grown plants was almost twice those in the green to red range. If this increase in weight of the shoots was due to the transport of nutrients and stored food from the cotyledons, then the weight of the cotyledons should have been relatively lower. This, however, was not the case as the weight of the cotyledons from the dark control was significantly higher than all the treatments except the yellow and white. The dry weight of roots from plants grown in the dark was significantly lower than those from yellow to the deep-red range. This rules out the possibility of the dark grown plants receiving more nutrients from the soil by means of a better root system. From these results it appears that photosynthesis contributed little to the production of dry matter in plants exposed to light. The differences in dry weight between the dark and light grown plants may be due to the imbalance between anabolism and catabolism. The low light intensities may have been stimulatory to respiration and probably the low rate of photosynthesis was unable to compensate for the energy loss. This could have changed the photosynthetic and respiratory quotients. Continuous monitoring of the O_2 and CO_2 in the cabinets might have given some insight into the reasons for these unusual results. There was a close relationship between growth measurements (table V) and dry weights (table VI). Plants which were tall and etiolated such as those of the dark, blue and red-far-red treatments had a significantly higher dry weight of shoots than those of the green to red treatments. As might be

expected the leaves of the dark grown plants, which were small and folded, had the least dry weight. In this connection it is interesting to examine the data for elongation of marked hypocotyl segments (table VII). It is obvious that essentially all the hypocotyl elongation occurred in the apical two segments. When compared to the light treatments the dark control plants had the least elongation in the ninth and the most in the tenth segment.

When the dark grown bean seedlings were exposed to 1 light cycle of the various wavelengths, and then returned to the dark, it was found that this had been sufficient to produce differences in elongation (fig 4). There was also a marked difference in the number of roots formed on hypocotyls excised from seedlings which had received 1 light cycle (fig 6, B). Therefore the effects of light may have been inductive in nature.

A number of workers (12, 21, 40, 67) have found that red light stimulates germination and far-red reverses this effect. In our study pigweed and stinkweed were found to be sensitive to light, having the highest germination in the orange (540 - 605 mμ) region. It was evident that far-red overlapping into the red region was enough to bring about inhibition of germination in these species. It was also observed that blue light was inhibitory to germination, which is in agreement with the findings of Flint and McAlister (21) for Lactuca sativa and of Wareing (77) for Nemophilia insignis. Wareing (77) suggested that the inhibition of germination by blue and far-red appears to involve the same photoreceptor. Although this is a possibility, the existence

of more than one photoreceptor should not be ruled out. Schulz and Klein (64) observed that several wavelengths, including far-red, red, blue and ultraviolet, were capable of suppressing germination and suggested that there may be more than one photoreceptor. They found no evidence for the existence of the phytochrome system. Wareing (77) observed the presence of an inhibitor in birch seeds, and demonstrated that light was necessary to overcome the effects of this inhibitor. It is possible that blue and far-red light induce the production of an inhibitor, which suppresses germination. Red light may convert the inhibitory substances into stimulatory or neutral compounds, which promote or allow germination. Hendricks, Butler and Siegelman (32) demonstrated that far-red light (730 mμ) converts P_{730} to P_{660} and thereby inhibits germination. On the other hand, red light (660 mμ) converts P_{660} to P_{730}, an active enzyme, which triggers germination.

In our studies the concentration of chlorophyll in the different light treatments followed very closely the absorption spectrum of chlorophyll. Virgin (74) found that red light was more effective than blue in inducing the development of chlorophyll. Our results with beans and normal barley substantiate his findings but are not in agreement with Appleman's (2) work on barley. Using light intensities approximately 7 times as high as those involved in this study he obtained a 33% higher concentration of chlorophyll in blue light than in red. In our studies the red treated plants had a higher concentration of chlorophyll than the red-far-red, which had about 60% of the amount in the red. This may indicate that the red-far-red system was operative in chlorophyll production as has been reported by Mitrakos (54) and by Price and Klein (61).

For corn and bean leaves the greatest amount of total nitrogen was found in the blue treatment. This finding is in agreement with the study done by Russian workers and cited by Tregunna (70). They found that the percentage of protein in leaf disks of corn, sunflower and beans was higher in blue than in red light treatments. Using $C^{14}O_2$ they reported that there was greater incorporation of radioactivity into the amino acids in the blue treatment than in the red. This was due mainly to an increase in amounts of alanine and aspartic acid. In our study, a comparison of amino acids in the acid soluble fraction of bean leaves showed that there were higher concentrations of alanine and aspartic acid, as well as histidine, threonine and serine in the blue than in the deep-red treatment. The acid soluble fraction is assumed to consist mainly of free amino acids. The dark and blue light treatments appear to result in either a greater production or less utilization of free amino acids. The high concentration of serine in the blue treatment is of special interest in this study, considering its importance in the biogenesis of tryptophan, a precursor of IAA.

A spectrophotometric method for estimation of IAA has been described in detail. The results of this study showed a relationship between height of plants and IAA content. Those light treatments which inhibited growth also lowered the endogenous IAA level. Although this association has been demonstrated, a cause-effect relationship has not been established. Recovery of applied labelled and unlabelled IAA was different in the various light treatments. Isotope studies showed differences in metabolism of IAA in the blue and red treatments. Less of the applied IAA was recovered from the red than from the blue

treated plants. Red light reduced elongation and dry weight of bean plants, but increased the leaf size, root formation and chlorophyll and carotene content. This trend was reversed in those treatments containing a higher proportion of far-red. It is inferred from our work that a number of these photomorphogenetic responses appear to be regulated by the phytochrome system. It was also observed that many of the plant responses in the red-far-red were similar to those obtained in the blue treatment. This similarity in response between the blue and red-far-red treated plants suggests that phytochrome may also absorb in the blue region or was not the only photoreceptor involved in the photomorphogenetic effects.

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